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BACTERIOLOGIC AND SEROLOGIC STUDY OF EIGHTY-NINE CASES OF DYSENTERY IN WHICH B. DYSENTERIAE FLEXNER AND B. DYSENTERIAE SONNE (B. DYSENTERIAE CASTELLANI-SONNE) WERE ISOLATED AS THE CAUSATIVE AGENTS*

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SHIGA in 1897 made the fundamental discovery of a small bacillus as the etiologic agent in dysentery. That year, during the progress of an epidemic in Japan, he isolated a short rod with rounded ends from the excreta, as well as from the mesenteric glands, of thirty-six fatal dysentery infections. This investigator[64] was undoubtedly the pioneer to resort to the use of serologic methods for the detection and identification of the causative agent of a disease. Literally working backwards, he regularly found in the feces of the afflicted an organism which, when cultured, was agglutinated by the blood serum of dysentery patients, while agglutination did not occur with (1) the serum of normal healthy individuals, (2) the blood serum of any patients affected by diseases other than dysentery, and (3) commercial antiserum prepared with unrelated organisms. He also demonstrated that human blood serum was capable of reactivating inactive antidysenteric horse-serum, and believed that the antidysenteric serum would find the proper complement when injected into the human body.[65] Shiga was undoubtedly the first to effect the immunization of horses by the subcutaneous injection of a saline suspension of killed dysentery bacilli which had been grown on agar.[66]

During the past three decades, because of the prevalence of this infection in

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various countries, the isolation of similar, but serologically unrelated organisms has been made by many investigators. Chief among these workers have been the following: Flexner,[24] Strong and Musgrave[72] working in Manila in 1900; Kruse[43] in Germany in 1900; Park[57] at Seal Harbor, Mt. Desert, Maine, in 1902; Duval and Bassett[19] at Baltimore in 1902; Duval and Shorer,[21] Wollstein,[79] Kendall,[38] Hiss and Russell[35] in 1903; Sonne[69] in Denmark in 1915; d'Herelle[32] in France in 1916; Øehnell[55] in Sweden in 1918; Mita[48] in Japan in 1921; Hoshi[36] in Manchuria in 1929.

Thjøtta[73] has divided the microorganisms studied by these investigators into three groups. The members of each being independent from the other two (1) in fermentative powers, and (2) in serologic reactions. The identification of these strains as the agents responsible for the major portion of this well recognized clinical condition has repeatedly confirmed Shiga's concept of bacillary dysentery.

A comprehensive review of the literature indicates that invasions with dysentery bacilli have been world-wide in their occurrence, and that epidemics were particularly numerous during the period from 1927 to 1932. Dysentery due specifically to the members of this group designated as B. dysenteriae Sonne have been frequently reported following the publication of Sonne in 1915.

During the last five years several cases of sporadic dysentery have occurred in this district. In 1929 during the months of August, September, and October a small epidemic (about forty cases) of infantile dysentery was manifested here. A relatively high percentage of those who were infected and less than one year of age succumbed to the disease. Organisms of the dysentery group, identified by cultural and fermentation reactions, were regularly recovered from the patients. As a control during the interval from January to July, 1930, the stools from 100 healthy infants were examined and no members of the dysentery group were isolated from any of the specimens. A similar study by Castellani[8] on normal individuals failed to detect organisms of this group under ordinary conditions.

The present paper is a report of an investigation of another more extensive and somewhat more acute epidemic of infantile dysentery which appeared in this community later in the same year, i. e., from July, 1930, to January, 1931. The incidence of the disease was greatest during the later part of August and throughout the month of September. (It is of interest that an epidemic of infantile diarrhea was present at the same time in Detroit, some 59 miles from Flint. Poole et al.[60] after a limited bacteriologic study were unable to satisfy themselves that members of the dysentery group were responsible for the epidemic.)

Eighty-nine cases were studied: 67 of these were hospitalized, and the remaining 22 were taken care of in their homes. Dysentery bacilli were recovered from 60 of these individuals. It was found that the germs which were isolated during the course of this investigation fell into three groups, and in the following pages they will be referred to as Types I, II, and III, respectively. It is to be noted that this designation does not correspond to the Types I, II, and III of Thjøtta. The following work was carried out to discover if possible the causative agent responsible for this epidemic, and it is believed that this objective was attained.

METHODS AND APPARATUS

The basic medium was prepared from "Lemco" meat extract. The liquid component was made by adding 5 grams of extract to each liter of water; to this solution was added 1 per cent Difco Bacto-Peptone and 0.5 per cent NaCl; the medium was then adjusted with N/1 NaOH to a reaction of P_H 7.6, after which it was boiled and filtered. Agar, 2 per cent, was added to the broth to be used in the preparation of agar plates and slants. The broth and agar mediums were placed in 15 by 150 mm. tubes and autoclaved at 110° C. for twenty minutes. To prepare the plates, the agar medium was liquefied by heat and then cooled to 45° C. before pouring into Petri dishes; about 12 c.c. quantities were placed in the dishes and then allowed to solidify, the water of condensation collecting on the surface of the agar was removed by evaporation before the plates were used.

Glassware.—All glassware was thoroughly cleaned and dried; tubes were plugged with cotton and subjected to dry heat sterilization before being filled with medium. Petri dishes (100 × 15 mm.) were wrapped in heavy manila paper and sterilized by dry heat.

Special Mediums.—For the fermentation tests six differential mediums were employed; glucose, lactose, sucrose, maltose, mannitol and rhamnose. Stock solutions of these substances in 20 per cent strength were prepared in distilled water; from these the final 1 per cent dilutions in broth were made and Andrade's indicator* was added to detect acid production. The hydrogen ion concentration of all mediums was determined with a Quinhydrone electrode, both before and after sterilization. These solutions were adjusted to a final hydrogen ion concentration of 6.8 with N/1 NaOH. Sterile Durham tubes (100 × 15 mm.) containing inverted vials (40 × 10 mm.) were then filled one-third. The tubes were subsequently placed in an Arnold sterilizer and heated for twenty minutes on three successive days. After inoculating into the various mediums, as a matter of convenience in reading the tests, the fermentation tubes were placed in open wire racks holding four rows of ten tubes each.

Litmus Milk.—The ordinary litmus milk was employed. A concentrated solution of litmus was added sufficient to color the milk a deep blue. This was sterilized by the fractional method.

Agglutination Technic.—In the serologic tests, tubes (75 × 10 mm.) of resistant glass were used. Copper racks containing three rows of ten holes each are convenient. Sterile graduated pipettes were selected in making the serum dilutions and in delivering the antigen suspensions. The antigens were grown on plain agar slants for eighteen hours. They were then washed with normal saline containing 0.4 per cent formaldehyde. The antigen suspensions were then filtered through sterile filter paper and made up to a standard density. The immune serums, Shiga, Hiss and Flexner, were commercial preparations,† and the Sonne immune serum was kindly supplied by R. L. Nelson. The serum dilutions (from 1:80 to 1:20,000) were prepared in sterile tubes (150 × 15 mm.) by the addition of saline. It is to be noted that these dilutions were used within thirty minutes after they were prepared.

*Andrade's Indicator was used in special fermentation reactions by Large in India, Perry and Bensted in Egypt, Johnston and Brown in Canada, and Welch and Mickle in the United States.

†These serums were obtained from Parke, Davis & Company.

The antigen suspension was added in 0.5 c.c. quantities to 0.5 c.c. volumes of the serum dilutions which had been placed in ten small test tubes (75 × 10 mm.). The tubes were then thoroughly mixed and shaken for two to three minutes, after which they were incubated at 37° C. for eighteen hours and the readings noted. A series of control tubes were made with the pedigreed antigens and studied simultaneously with the antigen suspensions of the organism isolated from the patient. The maximum titer of Type I, II, and III as well as the control antigens, are presented in Table II. Frequently there was no agglutination with the antigen in low dilutions of the homologous serum; while with the same serum in higher dilutions the antigen did give agglutination, the well known proagglutinoid zone phenomenon.

ISOLATION AND IDENTIFICATION OF ORGANISMS

Stool specimens were brought as soon as obtainable to the laboratory. Approximately 3 gm. of the suspected material was transferred into tubes (100 × 15 mm.) containing about 5 c.c. of sterile broth. The suspensions were thoroughly mixed by rotating the tubes between the palms of the hands for three minutes. These were then allowed to settle out at room temperature for fifteen minutes, thus giving a suspension that was free from large particles for preparing the dilutions.

A platinum loop (2 mm.) was used to transfer samples of the supernatant material. Two dilutions were made from each specimen as follows: Dilution 1 contained the 3 grams of infected material suspended in 5 c.c. of broth; from this Dilution 2 was prepared by transferring 3 loopfuls of Dilution 1 into 2 c.c. of sterile broth. A loopful of suspension was taken from each tube and placed upon the surface of a solidified agar plate and then spread by means of a sterile bent glass rod. By this method, following incubation the desired number of colonies, 25 to 100, was usually obtained on each plate.

The study of the growth appearing on the agar surface proved to be extremely interesting because of the variety of colonies present. It is noteworthy that plain agar medium[34] was used and gave excellent results in the differential study of the colonies, however, we ordinarily select eosin-methylene-blue or Endo's medium for this type of work because of the additional aids for identification. Observations were made after sixteen hours' incubation; the colonies being first examined by transmitted light, then against a black background (an improvised board covered with black paper). An ordinary hand lens was employed in this examination. Isolated colonies were marked and reexamined microscopically with the low power lens. Material was transferred with a platinum wire from the well separated colonies into lactose broth contained in fermentation tubes, and then incubated overnight. The preliminary use of this medium eliminated all lactose-fermenting strains. A loopful of the culture was removed from those tubes in which there was no acid or gas production and examined both in hanging drop and in stained preparations. Information regarding the size, shape, and structure of the cell was obtained in this manner and confirmed by the Gram stain. Those organisms which showed no acid or gas production in lactose broth were then inoculated into the following mediums:

glucose, lactose, sucrose, maltose, mannitol, and rhamnose contained in Durham tubes; after inoculation, they were incubated at 37° C. and readings were made at twenty-four-hour intervals for ninety-six hours. Controls with each series were made concomitantly both with stock laboratory strains and uninoculated tubes (to check the indicator) on all mediums employed (Table I). From the action of the organisms on the special mediums, they were divided into three distinct types. These special mediums although not as reliable as serologic tests are invaluable as a rapid diagnostic method for distinguishing between the various strains. "In fact, as justly remarked by Morgan, more varieties can be differentiated in this way than by using merely biological methods."[9] In order to have material available for further study, sub-cultures were made on plain agar before discarding the fermentation tests.

After the epidemic, during the months of December and January, the fermentation tests were repeated. The same routine was employed as in the original procedure, excepting that the readings were taken rather frequently (every twenty-four hours) for a period of three weeks. This method was used for the purpose of noting any delayed fermentation. Type II, which was isolated from seventeen cases, showed latent fermentation of lactose up to seven days. Characteristic readings were usually obtained with the other two types at the end of twenty-four hours (Table I).

TABLE I

COMPARATIVE DIFFERENTIAL REACTIONS OF DYSENTERY ORGANISMS

TYPES	NO. OF CASES	GLUCOSE 96 HR.	LACTOSE 96 HR.	SUCROSE 96 HR.	MALTOSE 96 HR.	MANNITOL 96 HR.	RHAMNOSE 96 HR.	LITMUS MILK 96 HR.
Type I	26	A*	–	–	*A*	A	*A*	AC
Type II	17	A	*A*[1]	A	A	A	A	A
Type III	17	G[2]	–	G	G	–	–	K
CONTROLS:								
Shiga[3]		A	–	–	–	–	–	AK
Hiss		A	–	–	A	A	–	AK
Flexner		A	–	*A*	A	A	*A*	K
Sonne[4]		A	A	A	A	A	A	A
Metadysentericus A[5]		A	A	A	A	A	–	AC
Ceylonensis A (A. D.)		A	A	A	A	A	A	AC
Ceylonensis A (S. H.)		A	A	*A*	A	A	A	AC
Ceylonensis A (P. D.)		A	A	A	A	A	A	AC
Ceylonensis B (R. W.)		A	A[1]	–	A	A	A	AC
Shiga[6]		A	–	–	–	–	–	AK
Hiss		A	–	–	A	A	*A*	AK
Flexner V		A	–	–	A	A	–	AK
Flexner W		A	–	–	–	A	–	K
Flexner X		A	–	–	A	A	–	K
Flexner Y		A	–	–	*A*	A	–	K
Flexner Z		A	–	–	A	A	A	K

*A, acid; *A*, variable (acid or negative); G, acid and gas. AC, acid and clotted; K, alkaline; AK, acid to alkaline.

[1]Tubes were frequently observed, ultimately (end of 168 hours) all produced acid in lactose.

[2]Tubes from four of this Type repeatedly produced gas in sucrose and maltose, as well as in glucose.

[3]These strains were received from American Type Culture Company.

[4]This strain was received from Nelson, R. L.

[5]These strains were received from Sir Aldo Castellani.

[6]These strains were received from Parke, Davis & Company.

CULTURAL AND MORPHOLOGIC CHARACTERISTICS

Method.—Plain agar plates were prepared and when solid and free from water of condensation a loopful of a young broth culture was transferred to the center of the plate and subsequently spread with a sterile bent glass rod. After sixteen to eighteen hours' incubation at 37° C. an examination of the plates showed the presence of three distinctly different varieties of colonies. It was extremely interesting to note that all of the strains belonging to any one of the three types based on the fermentation reactions produced similar colonies, therefore colony appearance was an additional means of separating the strains. The cultures were placed into three groups: Type I, II, and III on the basis of fermentation reaction, colony type and serologic response.

Type I.—All plates streaked with suspension of organisms belonging to this type showed on macroscopic examination round, glistening, moist, translucent

TABLE II

DIFFERENTIAL AGGLUTINATION REACTIONS OF DYSENTERY ORGANISMS WITH IMMUNE SERUMS

ORGANISM	NO. OF CASES	SHIGA ANTISERUM	NO. OF CASES	HISS ANTISERUM	NO. OF CASES	FLEXNER ANTISERUM	NO. OF CASES	SONNE ANTISERUM
Type I	1	–[1]	1	100	1	400	1	–
	5	–	5	–	5	1,000	5	–
	2	–	2	–	2	2,000	2	–
	2	–	2	–	2	5,000	2	–
	2	–	2	100	2	5,000	2	–
	2	–	2	200	2	5,000	2	–
	1	–	1	2,000	1	5,000	1	–
	2	200	2	–	2	5,000	2	–
	1	400	1	100	1	5,000	1	–
	3	–	3	100	3	10,000	3	–
	2	–	2	200	2	10,000	2	–
	1	–	1	1,000	1	10,000	1	–
	2	400	2	200	2	10,000	2	–
Type II	17	–	17	–	17	400	17	2,000
Type III	17	–	17	–	14	–	17	–
					3	400[2]		
CONTROLS:								
Shiga[3]		5,000		–		100		100
Hiss		–		200		1,000		–
Flexner		100		–		5,000		–
Sonne[4]		–		–		400		2,000
Metadysentericus A[5]		–		–		400		1,000
Ceylonensis A (AD)		–		–		400		2,000
Ceylonensis A (SH)		–		–		400		1,000
Ceylonensis A (PD)		–		–		400		2,000
Shiga[6]		2,000		–		–		
Hiss		–		200		–		
Flexner V		200		100		2,000		
Flexner W		–		–		5,000		
Flexner X		–		100		1,000		
Flexner Y		80		200		10,000		
Flexner Z		100		400		10,000		

[1]The titer is the highest dilution of the serum which caused complete or nearly complete agglutination of bacteria (3+ or better). – indicates negative.
[2]Type I was later isolated from these three cases.
[3] Numbers 3, 4, 5, 6. See footnote Table I.

bluish-white colonies of from 2 to 4 mm. in diameter. Upon further incubation the colonies increased in size. However, the edge was no longer regular, but became crenated. Microscopic examination after sixteen hours' growth showed short rods with slightly rounded ends, and these were decolorized by Gram's method. The average size of the cells was 1 to 3 microns in length and 0.5 to 0.8 microns in breadth. The organisms obtained both from the broth cultures and from the colonies on the agar plates were nonmotile. The general appearance of the colonies, the morphologic appearance of the bacterium, the fermentation reaction and agglutination results when compared with the controls and the published data of other workers indicated that this type belonged to the Flexner group (Tables I and II).

Organisms belonging to this type were recovered from twenty-six of the infections. Eight deaths occurred among the children under two years of age in this group. Type III, to be described later, was simultaneously isolated from four of these cases which had terminated fatally.

Type II.—These strains, Tables I and II, were identical in their fermentative and agglutinative reactions with the control cultures of B. dysenteriae Sonne. After sixteen hours' incubation the colonies were found to be round, smooth-edged, slightly opaque and moist, with a bluish tinge; and varied in size from 0.5 to 2.0 mm. On continued incubation all the colonies usually acquired a definite blue tinge, the surface being somewhat convex and the margin smooth and regular. On rare occasions larger colonies with irregular borders suggestive of R forms were observed but subcultures from these colonies gave the typical smooth S form. Microscopic examinations showed nonmotile, gram-negative rods varying from 0.8 to 2 microns in length and from 0.4 to 0.6 microns in breadth. Occasional involution forms were observed. Identification of these strains by their early production of acid in rhamnose and latent fermentation of lactose (three to seven days) classify them as B. dysenteriae Sonne (Table I). Serologic tests with this group are also comparable (Table II). Attention is called to the fact that Type II corresponds in its fermentation and serologic reactions with the strains of the Meta-dysenteriae organisms of Castellani (Tables I and II).

Clinical Observations.—This organism was isolated from seventeen cases, chiefly in children between one and seven years of age. In addition, four of these strains were recovered from the same number of nurses that had previously been on duty in the ward reserved for colitis patients. Moreover, in each of four families wherein there were two members attacked, the identical organism was isolated from each of the individuals. Indeed, it was noted that the second infection in the same family occurred approximately a week after the onset of the first. The microorganisms which are included in this group always caused conditions in which the clinical manifestations were sudden and severe. Bloody, mucous stools were passed frequently (6 to 15 a day), accompanied by a rapid elevation in temperature (102° to 105°). The blood count, which was not characteristic, varied from 12,000 to 18,000 white blood cells, with a corresponding increase in polymorphonuclear leucocytes and a decrease in lymphocytes. These symptoms usually ceased in seventy-two to ninety-six hours. No deaths were associated with the members of this group of organisms.

Type III.—The colonies on agar plates showed considerable variation in

size. They were round and convex, with a glossy mucoid appearance. On continued incubation the colonies increased in size and transparency, often coalescing to form a viscous mass. It would seem that the members of this group are mucoid variants[23] of Type I, since they fail to agglutinate in the presence of the antiserums of Shiga, Hiss, Flexner, and Sonne as well as homologous antiserums. This fact is in agreement with our observations* on the mucoid variants of B. para-typhosus A; B. psittacosis; B. icteroides and B. coli, however, we have been unable to dissociate these mucoid forms to S forms giving the characteristic reactions of the Flexner bacillus. The individual cells were actively motile, short, and plump, gram-negative rods. The growth of the members of this group in lactose broth was accompanied by the production of acid and gas (Table I). There was no agglutinative ability observed with any of the immune serums when used with these strains (Table II). The colonial growth was accompanied by a distinctly obnoxious, putrid odor.

Clinical Observations.—Strains belonging to this type were found in seventeen of the patients. In four of the fatal infections Type I was also present. There were five deaths in this group, four of which were associated with other conditions, as follows: three with malnutrition, and one with bronchopneumonia. The deaths occurred in infants less than one year of age.

ANALYSIS OF TABLES

In Table I may be found the reaction of all the cultures and controls on the special mediums studied. The strains of Type I show the characteristic fermentative reactions typical for the Flexner bacillus and were considered to be identical with this germ. The organisms gave the following reactions in the special mediums used: acid in glucose and mannitol, but variable in maltose and rhamnose; no change occurred in lactose and sucrose. Litmus milk became acid and coagulated. The fermentation reactions of Type II are seen to be identical with B. dysenteriae Sonne. Nine of these strains produced acid in lactose after four days, while eight did not produce acid until the sixth day. The reactions of the Meta-dysenteriae cultures obtained from Sir Aldo Castellani are identical with the fermentative reactions of B. dysenteriae Sonne. This suggests that the Meta-dysenteriaes of Castellani, the present Type II and B. dysenteriae Sonne are closely related organisms. However the colony formation of the Meta-dysenteriaes of Castellani closely resemble the variant mucoid form Type III. Type III fermentation reactions are identical with those given by typical strains of the Morgan bacillus.[49]

Upon reference, in Table II are given the maximum agglutination titers of the Flexner serum with strains of the three types isolated as well as with pedigreed cultures. Type I was isolated from eight of the fatal infections, which gave the following agglutination results:

TYPE I

3 strains	agglutinated	Flexner Immune Serum	1:10,000
2 strains	agglutinated	Flexner Immune Serum	1: 5,000
1 strain	agglutinated	Flexner Immune Serum	1: 2,000
1 strain	agglutinated	Flexner Immune Serum	1: 1,000
1 strain	agglutinated	Flexner Immune Serum	1: 400

*Soule, M. H., with Heyman, Anne M.: (In press).

Type II was agglutinated by B. dysenteriae Sonne immune serum 1:2,000 and by Flexner immune serum 1:400. In a similar series of agglutination reactions the Meta-dysenteriaes of Castellani also gave very comparable results. From the results obtained by the use of the Meta-dysenteriae cultures as controls in our investigation the biochemical and the serologic reactions, which are given in Tables I and II, indicate that these organisms are identical with B. dysenteriae Sonne, isolated by Sonne in Denmark in 1915. This fact is also in accordance with the results obtained by Cerruti in a report by Castellani[12] and by Koser et al.[42] If we are to accept the criteria established by Shiga (i. e. the identification of microorganisms by serologic methods), we must recognize that the organism discovered by Sonne in 1915, and classified by Thjøtta as Type III, is the same organism found by Castellani in Ceylon in 1904 and in 1905.[6, 7] It is suggested that this organism be reclassified as B. dysenteriae Castellani-Sonne. Investigations by Cerruti[14] confirm this opinion.

It is not clear as to whether the cultures used by Koser et al.[42] under the designation of Ceylonensis A and Ceylonensis B are the same strains included in the present study under the same names. Our strains were kindly furnished by Sir Aldo Castellani as already indicated. Differences in the fermentative power of Ceylonensis A and Ceylonensis B similar to those observed by Koser were noted indicative of the fact that they do not belong to the same group of organisms and the terminology is therefore confusing.

The serologic reactions of Type III were unsatisfactory, only three strains showed agglutination by Flexner immune serum at a dilution of 1:400. From the patients infected with those three strains Type I was later isolated. Morgan and Ledingham[50] found that the agglutination results of B. morgani were not specific. Kligler[40] in a study of seventeen strains of B. morgani found them culturally identical but highly diversified antigenically. "While no conclusions can be drawn regarding the pathogenic significance of this bacillus, the wide diversities of antigenic properties raise the question as to the specific relationship of the various cultures met with as well as their relation to a definite class of pathologic processes in man."

The question frequently arose as to whether or not the strains placed in Type III were B. coli. The cultures were carefully examined with this consideration in mind but all of the data indicated that such was not the case. It would have been very desirable to compare the reactions of these strains simultaneously with known cultures of B. morgani, but such experiments were not carried out.

In Table III are summarized the clinical data of the 89 cases of dysentery

TABLE III

CLINICAL DATA OF DYSENTERY

NUMBER OF CASES	AGE	DEATHS
64	6 weeks to 2 years	17
20	2 years to 8 years	0
4	18 years to 21 years*	0
1	27 years**	0

*Student Nurses with Type II (Sonne).
**Mother of Infant with Type III (Unclassified).

studied. Although there were 17 deaths, 7 of these were associated with other diseases. Hospital charts were carefully examined. An increase in temperature was noted in every instance, the maximum curve in each case ranging from 99.6° to 105°. The blood count was not indicative, although there usually appeared an increase in the white blood cell count which was accompanied by an increase in the polymorphonuclear leucocytes and a decrease in the lymphocytes. The red blood cell count appeared normal, excepting in cases of extreme dehydration. The stool specimens obtained varied from four up to twenty per day. Some contained blood and mucus, while others were watery, green, with small amounts of mucus. Vomiting occurred in about 50 per cent of these cases. The average length of illness was approximately fourteen days. The aforementioned data were gathered from the 67 infections which were hospitalized.

DISCUSSION

Type I.—Type I, which is seen to agree serologically with B. dysenteriae Flexner, was found most frequently in this epidemic. Bacteriologic, fermentative, and serologic identification of this type was obtained in 26 infections, and 8 of these proved fatal. In 3 of the fatal infections this germ was isolated from the mesenteric glands at postmortem examination. Duval and Bassett[20] also obtained this organism from the mesenteric glands and liver of infants after death, and Kinloch[39] in Scotland also obtained the Flexner bacillus from the intestine of a two-year-old child at autopsy. Fatal infections by this microorganism, especially in children have been reported by the following investigators: Flexner,[25] Duval and Bassett,[19] Park,[57] Wollstein,[79] Davison,[18] and Silverman[67] in the United States; Rajchman and Western[61] in the Eastern Mediterranean District; Maitra[46] in the Jheria Mining Settlement in India; Kinloch[39] (1919) in Scotland; Webster and Williams[77] in Australia; Warren[76] in England; Rosenbaum[63] and Rimpau[62] in Germany; Auche and Campana[3] in France; Torres-Umaña[75] of Bogotá; Padua[56] in the Philippines. Although infections with these organisms are usually associated with infancy, some of the cases reported by the above investigators have been in adults. Wollstein,[79] in a report of 39 positive infections of infantile diarrhea in 1903, in which 29 infants died, found that the causative agent agglutinated with Flexner immune serum in a dilution from 1:50 to 1:3,000. Again, in a second study, in 1922,[80] of 86 infections, 20 of which were positive she found that the agglutination reactions of the mannite-fermenting strains, which were isolated, to be high with Flexner polyvalent serum, i. e., 1:3,200 to 1:6,400. The significant factor in these results is that the highest agglutination titer was obtained with polyvalent serum. Manifold and DeMonte[47] investigating outbreaks of dysentery among the troops in India, isolated B. flexner as a causative agent. The agglutination results obtained by these workers with stock laboratory Flexner immune serums against their Flexner bacilli varied from dilutions of 1:500 to 1:5,000. The data obtained with their polyvalent immune serums are comparable with the serologic results given by our Type I with Flexner polyvalent immune serum, which varied from 1:400 to 1:10,000 (Table II).

The wide distribution of this organism and its association with a high mortality rate in children is worthy of note.

Type II.—Strains of this type which were isolated from seventeen of the dysentery infections, possess the morphologic, cultural, fermentation and serologic characteristics of B. dysenteriae Sonne and excepting colony type the Meta-dysenteriaes of Castellani. Sonne[70] in 1915 by repeating the classical serologic experiments of Shiga, was the first to demonstrate that this type was serologically specific and independent of other bacilli of the dysentery group. He also showed that this was the causative agent in a series of infections in Denmark.[71] d'Herelle,[33] without the knowledge of Sonne's work, isolated and described the same organism again in 1916. Since then this microbe has been found by the following workers in several countries: Thjøtta[73] in Norway; Øehnell[55] in Sweden; Mita[48] and Kobayaski[41] et al. in Japan; Patterson and Williams[58] in Australia; Andrewes,[1] Nabarro,[51] Smith,[68] Channon,[15] Evans,[22] Clayton and Hunter,[16] Clayton and Warren,[17] Cann and Novasquez[5] in England; Fraser, Kinloch and Smith,[26] Fyfe,[27] Hay[30] in Scotland; Perry and Bensted[59] in Egypt; Large[44] in India; Buchanan and Roux[4] in South Africa; Johnston and Brown[37] in Canada; Gilbert and Coleman,[28] Nelson,[53] and Welch and Mickle[78] in the United States.

Duval and Shorer,[21] and Kendall[38] in 1904 noted atypical fermentation reaction, i. e., slow-lactose fermenting organisms, which also gave a low agglutination reaction with polyvalent immune Flexner serum. The organisms giving these reactions were classified as atypical Flexner bacilli. It is conceivable that these strains are the same organism as that identified by Sonne in 1915. However, in 1904 and 1905 Castellani isolated two organisms at Ceylon[6, 7] which he described in 1907 under the names B. ceylonensis A and B, ceylonensis B, the former being very similar to the Sonne bacillus. In 1927[10] and 1932[11, 12] Castellani classified these two organisms and other closely related forms as "Meta-dysenteriae Bacilli," to identify those members of the dysentery group which produce acid slowly in lactose. The cultures of Meta-dysenteriae received from Castellani, and used as controls in this investigation, have proved to be identical in their fermentation and serologic reactions with B. dysenteriae Sonne obtained from Nelson (Tables I and II).

It is to be noted that the members of this group in contrast to infections with Flexner's bacillus were never, of themselves, able to cause the death of the patient. However, Evans[22] in England and Hay[31] in Glasgow report B. dysenteriae Sonne isolated as the causative agent in three fatal cases. It is indeed interesting that most workers have isolated this microorganism from children between one and ten years of age. In the present epidemic, as noted, Sonne infections occurred chiefly in children less than eight years of age, and none of these terminated fatally. Typical photographs of the colonial growth of this organism may be found in articles by Thjøtta,[73] Kobayaski et al.,[41] Cann and Novasquez[5] and Welch and Mickle.[78] The characteristic delayed fermentation of lactose serves admirably as a means of identifying this organism from other members of the dysentery group.

Since Shiga's classical agglutination studies, the clumping of an organism by its specific antiserum has been held as a final criterion in the identity of the microorganism. In reviewing the literature it was noted by Gilbert and Coleman[28] that B. dysenteriae Sonne gave agglutination in low dilutions with poly-

valent immune dysentery serum. Patterson and Williams[58] in Australia found that the reactions with Flexner polyvalent immune serum versus the Sonne bacillus gave agglutination of 1:400, while with the homologous serum, they obtained agglutination in dilutions of 1:2,400. Perry and Bensted[59] working in Egypt have reported the following: "In the serological investigation of the strains isolated, a few of the organisms were agglutinated to a low titer by a polyvalent serum prepared in these laboratories from type strains of B. dysenteriae Flexner V, W, X, Y, and Z." And further, "When titrated against a Sonne serum prepared from classical strains of this organism that had originally been obtained from the National Collection of Type Cultures, all the strains recovered were agglutinated to full titer." The fact that the Flexner species possesses antigenic relationship with many other species has been shown by Andrewes and Inman[2] (1919). Clayton and Warren[17] (1927), confirmed this antigenic relationship which was originally demonstrated by Andrewes and Inman. Gilbert and Coleman[28] (1929), reported the following: "In polyvalent dysentery immune serum prepared with the non-lactose fermenting strains, cultures of the Sonne dysentery bacillus are usually agglutinated only in low dilutions." The strains of Type II, isolated in the present study confirm the observations of these investigators, suspensions of the organisms were found to agglutinate regularly with Flexner polyvalent immune serum in a dilution of 1:400 (Table II). Large[44] in the Lahore District in India reported that Type A Sonne will agglutinate in Sonne serum in high titer; Nabarro[52] in England, Johnston and Brown[37] in Canada, Welch and Mickle[78] in the United States reported agglutination from 1:1,280 to 1:2,560 on most occasions with homologous serums in infections from which the Sonne bacillus was isolated. Strains of Type II in the present studies gave an agglutination with Sonne immune serum of 1:2,000 (Table II). Hay[31] reported an agglutination of 1:1,600. The serologic results obtained both with polyvalent Flexner serum and the homologous Sonne serum by these workers are comparable with those obtained with strains of Type II (Table II) from seventeen infections of dysentery. Attention is likewise called to the results given by Welch and Mickle,[78] since they also employed Sonne immune serum from Nelson. These workers report that the Meta-dysenteroides strain obtained from the American Type Culture Collection was agglutinated by the Sonne immune serum from Nelson in high dilutions, i. e., 1:2,560. The specific serologic reactions of the Meta-dysenteroides strain suggest the identical relationship between this group and B. dysenteriae Sonne. The reports of many investigators show that in infections from which B. dysenteriae Sonne have been obtained suspensions of the organisms gave, in many instances, a low agglutination titer with polyvalent Flexner immune serums, while with homologous serums the agglutination titer varied from 1:1,280 to 1:2,560. In the present study the Meta-dysenteriae cultures of Castellani were agglutinated by Nelson's Sonne immune serum as follows: Two cultures 1:1,000, and two cultures 1:2,000 (Table II). Cerruti[14] in 1930 observed the agglutination of these same strains from Castellani by Sonne serum.

Nelson,[54] Koser et al.[42] prepared immune serums having an agglutination titer of over 1:5,000 when used with strains of the Sonne organism obtained from various sources. These serologic tests confirm Sonne's researches which demon-

strated his organism as having a very definite serologic specificity. The fermentation and agglutination reactions obtained with the strains of Type II identify them as the Sonne bacillus and as is indicated were isolated from seventeen of the infections.

The recent serologic studies of Thjøtta and Waaler[74] on the S and R forms of dysentery bacilli Type III have an important bearing on the identification of this organism by serologic methods. In their investigations these workers found that the dissociants were agglutinated by their homologous antiserums at significant titers (1:640) but that the organisms in the presence of the heterologous serums were not agglutinated at titers possessing diagnostic value (1:40). It is also of interest to record that in no instance during the present study were so-called R. forms of the dysentery bacillus encountered although their possible presence was always borne in mind when examining the plates.

Type III.—Type III remains unclassified. Although the fermentation reactions of these strains were consistent with those reported for the Morgan bacillus by other workers no serologic relationship with any of the antiserums employed could be demonstrated. Morgan and Ledingham[50] in 1909 reported that the agglutination results of B. morgani isolated in 1906 are not specific, Kligler[40] in a study of seventeen strains of B. morgani found they were culturally identical, but antigenically they were highly diversified. "While no conclusions can be drawn regarding the pathogenic significance of this bacillus, the wide diversities of antigenic properties raise the question as to the specific relationship of the various cultures met with as well as their relation to a definite class of pathologic processes in man." Havens and Ridgway[29] in 1929 refer to the Biologic Relationship of Morgan's Bacillus as follows: "While it is not clear that agglutionogenic differences observed between strains of Morgan's bacillus can be explained on the basis of mutation, the results are roughly comparable to those obtained with certain strains of B. typhosus, which showed agglutinogenic differences but were identical in complement fixing properties." "Complement fixation reactions between ten strains of B. morgani revealed close antigenic identity. The serologic unity demonstrated by this method permitted a study of antigenic relationship of B. morgani to members of the typhoid-dysentery group. Close relationship was indicated to the paratyphoid and paradysentery strains studied." Indeed, MacKenzie and Batt[45] found in an antigenic analysis of B. morgani, which had been isolated in an epidemic of summer diarrhea, that this organism usually was not agglutinated by the polyvalent serums employed. However, they found that B. morgani was agglutinated by polyvalent immune horse-serum in a dilution of 1:80. This agglutination of B. morgani with immune polyvalent serum is much lower than that obtained with microorganisms of the dysentery group under the same condition. They arrived at the conclusion that "B. para-dysenteriae isolated during the epidemic has been shown to contain an antigenic component which is also present in one of the cultures of B. Morgani." However, in a very recent report of Castellani and MacKenzie[13] of nine strains of B. morgani No. 1 which were identical in their fermentation reactions, five different serologic types were separated. They found, moreover, that the serum prepared from the culture of the organism, by intravenous injections into the ears of rabbits, agglutinated its homologous organism in most cases 1:800 and 1:1,600.

In the present investigation of the nonagglutinability of strains of Type III (Table II) by the immune serums employed it is suggested that these strains may be variants of Type I or Type II. However, the fermentation and serologic results with strains of Type III are comparable with those reported by MacKenzie and Batt for B. morgani.

SUMMARY

1. Eighty-nine cases of an acute epidemic of diarrhoeal disease are reported. Eighty-four of the patients were under eight years of age and of this group, 64 were less than two years old.

2. Organisms belonging to the dysentery group were isolated from sixty of these infections.

3. A detailed study of the morphologic, cultural, fermentative and serologic reactions of the various strains isolated is presented.

4. The cultures on the basis of these reactions are classified into 3 groups.

Type I appears to be identical in its fermentative and serologic reactions with B. dysenteriae Flexner. This type was found most frequently during the epidemic, occurring in 26 cases, eight of which terminated fatally. *Type II* corresponds, colony formation excepted, to B. Meta-dysenteriae Castellani and is identical with B. dysenteriae Sonne. Members of this group were isolated from 17 cases in which the clinical manifestations were severe, but in contrast to infections with the Flexner type, were not fatal. *Type III*, based on fermentation reactions, the members of this group are very closely related to B. morgani. However from the observation that this type was present in four of the fatal infections in which the Flexner organism was the causative agent, it is considered that Type III is a variant of Type I. Considerable experience on the part of the authors with the mucoid variants of the members of the colon-typhoid-dysentery groups of organisms lends further support to this view.

5. The data indicate that there were two definite serologic groups, B. dysenteriae Flexner and B. dysenteriae Sonne, among the three types isolated and that they were the causative agents of the present epidemic of infantile dysentery.

6. On the basis of priority, it is suggested that the name B. dysenteriae Castellani-Sonne be given to the members of the Type III dysentery organism of Thjøtta.

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STUDIES ON THE ETIOLOGY OF POLIOMYELITIS: ISOLATION AND CULTIVATION OF AN ORGANISM AND TRANSMISSION OF THE DISEASE IN MONKEYS*

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INTRODUCTION

LESS than fifty years have elapsed since Strümpell[1] in Vienna first hinted that poliomyelitis, described as a clinical entity by Jacob Heine[2] in 1840, was probably an infectious disease. Medin,[3] reporting an epidemic in Sweden in 1889, strengthened the idea that paralysis was but one feature of an acute general infection. The modern conception of the disease was developed only a little over twenty-five years ago by Wickman,[4] who in 1905 contributed a monumental work on that great epidemic in Sweden. His epidemiologic studies also added convincing proof of the infectious nature of poliomyelitis and its communicability.

The numerous pathologic studies beginning with Provost and Vulpian[5] in 1865 and culminating in the classical works of Flexner and his associates[6] gave a clear picture of the course of the disease and its far reaching effects in the animal body.

These newly acquired facts served all the more to stimulate a widespread search for the causative agent. In the attempts that followed there was opened a wide field of investigation from which came a more detailed knowledge of the disease. Signalizing the experimental approach to the problem was the first successful transmission of poliomyelitis to monkeys accomplished almost simultaneously in Vienna by Landsteiner and Popper[7] and in the United States by Flexner and Lewis.[8] To the last named, however, belongs the credit for the most fundamental achievement whereby the infection was propagated indefinitely through series of animals by means of direct inoculation of the virus into the brain. For, with regard to such crucial experiments, the European investigators had not been successful. The infecting agent was subsequently found to be capable of passing through the pores of a Berkefeld filter. Flexner and Lewis[9] in 1909 and Landsteiner and Levaditi[10] in the following year thereby added poliomyelitis to the known diseases caused by active agents smaller than ordinary microorganisms, and hence known as filterable viruses.

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ARTIFICIAL CULTIVATION OF FILTERABLE VIRUSES

Numerous attempts have been made to cultivate viruses in general and that of poliomyelitis in particular. It is not inconsistent with the nature of invisible things that there be some discussion as to their properties or to their existence in fact. Certain filterable viruses, much smaller than ordinary bacteria, are conceded to be in all probability optically immeasurable. Curiously, the greatest amount of discussion hinges about those viruses that are not quite so small, yet are capable of passing through different kinds of filters.

Observations made in the past have led to the formulation of certain principles concerning viruses. Some of these statements are perhaps open to question and in the light of newer bacteriology would appear to merit some revision. It has been said that living tissues or cells are indispensable for the growth of etiologic agents of the nature of filterable viruses. Opposed to this is the fact that viruses, none the less, have remained invisible in living tissue. Given the proper chemical and nutritive basis in a culture medium, it should be possible for virus to multiply in tissue containing nonliving cells. Another moot question has concerned itself with whether or not the viruses multiply intra- or extracellularly. This has led to an involved literature on the so-called "inclusion bodies." These are said to be the products of degeneration by some, by others they are considered as the virus itself or as the evidence of its activity, and finally as having no specific characteristic. At the moment although their exact nature is uncertain they are not accepted generally as stages in the life cycle of an organism.

These questions may be answered perhaps by observations that have been made upon filterable forms of bacteria. Any discussion of filterability must entail all phenomena of bacterial growth as well as that of virus multiplication. The more recent experimental studies have indicated the importance of complex metamorphoses and life cycles in the growth and development of organisms hitherto believed to be stable and simple. These observations are but reaffirmations of the well grounded pioneer studies of Lankester,[11] Löhnis,[12] and others. Pleomorphism has been viewed with much skepticism in matters pertaining to the etiologic significance of microorganisms. The occurrence of metamorphosis and a life cycle has been conceded to protozoal forms but has been denied bacteria and viruses. The newer bacteriology appears to have broken away from this point of view.[13, 14, 15, 16] Noteworthy contributions in the field of filterable viruses have been numerous, among them the more recent reports of Rivers,[17] Zinsser[18] and associates,[19] Cowdry,[20] Olitsky,[21] Goodpasture,[22] Noguchi,[23] Flexner,[24] and Shope.[25]

EARLIER STUDIES IN THE CULTIVATION OF POLIOMYELITIC VIRUS

The original paper of Flexner and Noguchi[26] and the subsequent reports of Amoss,[27] Smillie[28] and Heist and Kolmer[29] defined the characteristics of the "globoid bodies" cultivated from tissues of the central nervous system from human and experimental sources. Certain definite criteria of the cultures included the essential requirement of an anaerobic culture medium containing a piece of fresh sterile rabbit kidney and ascitic fluid. In respect to pathogenicity it was found that only exceptional cultures were infectious and that saprophytic strains were cultivable with difficulty while parasitic ones were even more refractory. Olitsky,[30] repeating these studies, was unable to obtain satisfactory secondary growths in later subplants and characterized these results as disappointing. In these experiments it should be noted that a Berkefeld V filter was used in the preparation of filtrates from the brain and spinal cord as well as

fragments of these tissues. Next he applied the method of cataphoresis to the contents of the culture tubes and was unable to differentiate "microorganisms" from precipitate. This procedure was based on the previous observations made by Olitsky,[31, 32] that poliomyelitic virus in suspensions of nervous tissue migrated to the anode. The power of such "cultures" to infect and induce experimental poliomyelitis was then studied in monkeys. He employed suspensions of anodic material obtained by cataphoresis of poliomyelitic nervous tissue from a monkey and successfully produced the disease in one animal. In one series of subplants derived from the brain and cord of this infected monkey, materials taken from "cultures" in the seventh to the tenth transplants produced experimental poliomyelitis. Prodigious numbers of "globoid bodies" recovered from as many as twenty to thirty or more tube cultures, were inoculated.

From all the available evidence and the conclusions drawn by Olitsky it would appear that the minute particles did not represent true cultures of the globoid bodies or of any living organism. He has suggested the hypothesis that the virus has been adsorbed to the minute particles seen in these "cultures" and which resemble closely the "globoid bodies" of Flexner and Noguchi. Olitsky has further suggested the possibility of the virus having multiplied in a modified living tissue-cell culture.

EXPERIMENTAL

In the investigation to be reported we had access to material taken from monkeys that had been infected experimentally with an active strain of poliomyelitis virus obtained from the Rockefeller Institute through the courtesy of Dr. Flexner. Two additional strains were obtained from other institutions and represented original Flexner virus that had been used successfully in the production of experimental poliomyelitis.

Macacus rhesus monkeys were inoculated* intracerebrally with 0.2 to 0.5 c.c. of a 5 per cent saline Berkefeld N filtrate prepared from the brain and cord. After definite paralysis had occurred following a typical clinical picture of the disease, the animals were etherized and the brain and spinal cord removed with strict sterile precautions. The tissues were tested for bacterial growth before and after storage in sterile 50 per cent glycerol. This glycerolated material in the primary cultivation experiments was used for inoculation of media after five to six months' preservation in the ice chest at a temperature of 4° C. In the recultivation experiments the materials prepared from the brain and cord of monkeys that had been infected by means of cultures were inoculated into media on the same or on the following day. Such tissues had been preserved in glycerol at ice box temperature for periods ranging from four hours to one to four days, and a maximum period of fifty-four days (Monkey 38).

Culture media was inoculated with 0.1 to 1.0 c.c. of a Berkefeld N filtrate of a 5 per cent suspension of the brain and cord for primary cultivation. Both the filtrates and suspensions separately of brain and cord, respectively, in amounts of 1.0 c.c. were used in the recultivation experiments from monkeys successfully infected with cultures.

CULTIVATION†

Seven different strains of poliomyelitis virus were studied. "EB" medium to be described later was used as the original substrate from which subplants were made to the "VB" medium. The first cultures were successfully isolated according to the procedure to be reported in the text. For the recultivation of organisms

*All inoculations in monkeys were performed under ether anesthesia and with strictest surgical asepsis.

†For revised technic and details, refer to Proc. Soc. Exper. Biol. & Med. **30**: 92, 1932.

from monkeys that had been successfully inoculated with the cultures it was found that the "VB" substrate alone seemed to be satisfactory.

The culture medium consisted of sterilized minced sheep brain contained in a veal infusion broth free from peptone. It was thought highly desirable to avoid the use of fresh living tissue and ascitic fluid such as characterize the Noguchi medium or its modifications. Another departure from the usual technic concerned the oxygen requirements during incubation. No attempt was made to obtain strict anerobiosis by means of the recognized methods. The reduced oxygen tension and anerobiosis developed in the medium were found satisfactory. The use of brain tissue suggested itself as a suitable culture medium for a virus that is known to exhibit a marked affinity for the nervous system.

Minced brain medium which will be referred to as VB was prepared in the following manner:

The blood and membranes were carefully removed at autopsy from fresh sheep brain. One kilogram of tissue was added to one liter of veal infusion broth, P_H 8.0, free from peptone. The mixture was coagulated thoroughly by boiling for two to three minutes and 0.2 per cent glucose by volume was added. The cooled material was then passed two or three times through a meat chopper. The culture medium was sterilized in small flasks at 15 pounds' pressure for twenty minutes and stored. Test tubes measuring 1.5 by 15 cm. were filled to a depth of approximately 6 cm. (or about 10 c.c. by volume) and sterilized in the autoclave at 15 pounds' pressure for twenty minutes. The tubed medium was sterilized a second time at 15 pounds' pressure for ten minutes. The final P_H was adjusted (if necessary) to 7.6. This reaction underwent no appreciable change in the course of prolonged incubation of the cultures in the medium owing to its natural buffering quality.

The following experiment was made to determine the degree of heat penetration necessary for proper sterilization, and to rule out the possibility of living tissue in the culture medium.

A maximum recording thermometer was placed in the center of the medium contained in a test tube and held firmly in position 2 cm. from the base. The pressure within the autoclave was raised to 15 pounds and within five minutes the temperature registered 118° C. After exposure of three minutes to this temperature, the steam was shut off and the autoclave opened two minutes later. The temperature within the tube of medium, at this time, registered 96° C. The tube was allowed to cool spontaneously, and thirty-five minutes after the beginning of the experiment the temperature within the tissue mass registerd 68° C. From this experiment it is evident that an exposure of fifteen to twenty minutes at 15 pounds' pressure would be sufficient to destroy any living tissue and to effect complete sterilization. Resistance of nervous tissue to high temperatures is considerably less than that of other tissues. Pincus and Fischer[23] found them to be extremely labile following exposure of a few minutes to 60 or 65° C.

Tests for sterility were made by incubating the medium at room temperature (20° to 24° C.) and at 37.5° to 38° C. for three or four weeks. As an additional precaution 3 or 5 c.c. samples of the medium were transplanted to simple and enriched culture mediums and carefully observed for three or four weeks to rule out all possible sources of contamination.

The inoculum, consisting of either filtrates or suspensions as already described, was next added to a series of 8 to 10 tubes of culture medium and incubated at 37.5° to 38.0° C.* Two tubes in each set were left unopened during the three or four weeks' period of observation during which samples from the others were carefully withdrawn for subplants every week or ten days by means of a platinum loop or a sterile capillary pipette (approximately 0.1 c.c.) and for film preparations. Control tests included Berkefeld filtrates and suspensions of poliomyelitic monkey brain and cord inoculated into ordinary simple fluid and solid culture mediums; filtrates and suspensions from normal monkey brains and from herpes, encephalitis and neurovaccinia; and finally uninoculated "VB" medium.

Subplants from tubes that yielded growth were made every five to ten days. The second and subsequent generations were found to developed satisfactorily within a week. The un-

*32°-34° C. has been found more satisfactory.

opened reserve tubes were examined after growth had occurred in the remaining series and were found to contain the identical organisms.

Subplants from the tubes that showed primary growth with the inoculum were made by means of a single 2 mm. platinum loopful for the first three or four transfers. This was done in order to effect many millionfold dilutions of the original virus. At the same time transfers were also made to ordinary brain-heart (Bacto) infusion broth and blood agar plates.

A modified technic was also used for cultivating the virus and maintaining culture subplants in parallel series with VB medium. The method of Kendall[34] was followed in part in

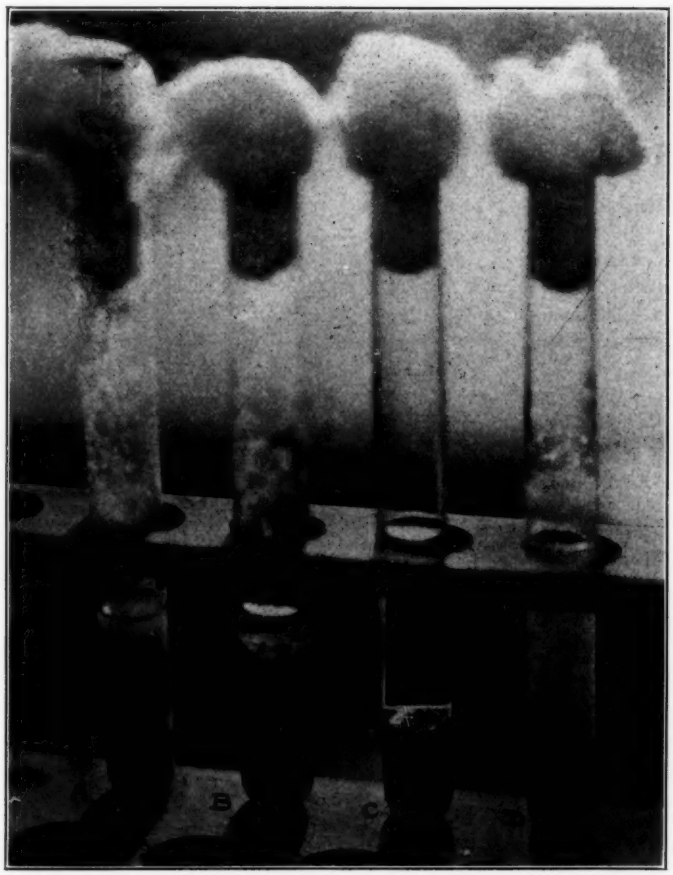

Fig. 1.—Culture medium "VB" (tubes A and B), and "EB," (tubes C and D). Positive growth in tubes A and D, showing slight turbidity, uninoculated controls B and C, remaining clear, with sharp line of division between tissue and fluid. The changes due to microbial growth can be seen on the surface of the column of brain tissue immediately beneath the supernatant fluid.

the preparation of granulated sheep brain substrate to be referred to as EB medium. The "K" medium (hog intestine) of Kendall and sheep brain prepared according to his method and used in the manner suggested by him for the "K" substrate gave negative cultivation results in our study. Hence the extraction procedures were modified somewhat and the final concentration of brain tissue in 0.85 per cent sodium chloride was made up to 10-20 per cent by weight instead of 2 per cent. The final P_H was adjusted to 7.6 instead of 7 to 7.4 and the temperature of incubation was 37.5° to 38.0° C. instead of 30° C. as recommended by Kendall. Our procedure was as follows: Sheep brain free from blood and membrane was ground finely in a meat chopper. The material was extracted once with two volumes of 95 per cent ethyl alcohol* and the dry tissue residue was next extracted in the same manner twice with two volumes of U. S. P. benzol. The

*Twelve to sixteen hours at 20-22° C.

resulting finely powdered dry material added in the proportion of two grams to 10 c.c. of 0.85 per cent sodium chloride solution, was mixed thoroughly and sterilized in the autoclave at 15 pounds' pressure for twenty minutes. In our hands this medium appeared to offer no special advantage in carrying on the culture transplants. The complicated technic of preparation also discouraged the use of this medium. Whether or not this substrate is essential for the successful cultivation of the virus in conjunction with the VB medium remains to be determined.

Essentially the procedure was to inoculate the virus material into the EB medium and to make subplants at suitable intervals to the VB substrate in which the cultures were maintained by serial dilution. In a number of instances successful growth was obtained by means of the VB medium alone. Undoubtedly the two mediums exerted different effects upon growth, morphology, and virulence. Reversible morphologic changes were unusually striking when observed over long periods of time.

Tubes found to contain ordinary bacteria that might be contaminants were discarded. Generally these could be detected after twenty-four to forty-eight hours of incubation. Cultures that gave macroscopic evidence of growth only after fourteen to twenty-one days were set aside for study and subplanting. At such times a definite faint turbidity changing to unmistakable clouding of the culture medium took place in the deeper levels of the supernatant layer of fluid in the tubes containing VB medium. The brain tissue immediately beneath this fluid was now covered with a fine grayish-white layer of material. The control tubes remained entirely clear during this period and ruled out the possibility of tissue autolysis causing the turbidity observed in the inoculated tubes (Fig. 1). A sterile platinum loop applied to this layer of material brought away a slightly viscous fluid adhering momentarily by a fine thread as the loop was withdrawn.

Film preparations were allowed to dry on slides *without heat fixation* and were stained by the methods of Wright, Gram, and Giemsa. It is noteworthy that the first generation cultures of filtrates from several strains of poliomyelitis virus yielded a definite turbidity in the EB culture medium without revealing organisms in numerous film preparations. Subsequent transplants to the VB medium or to corresponding EB medium, however, were found to contain living organisms. These were absent in the control culture tubes inoculated with material from normal monkey brain and the viruses of herpes, encephalitis, and neurovaccinia. Subplants from apparently negative EB medium tubes showed growth microscopically in the VB medium as early as ten to fourteen days before this could be demonstrated in the original culture tubes (Table I).

TABLE I

SUMMARY OF CULTURE SUBPLANTS OF POLIOMYELITIS VIRUS (FIRST THREE GENERATIONS)

DATE OF INOCULATION	GENERATION	CULTURE MEDIUM	DATE OF POSITIVE GROWTH
8/19/31	1	EB	9/14/31 (Negative 9/10/31)
8/28/31	2	VB	9/4/31
8/28/31	2	VB	9/10/31
8/28/31	2	EB	9/14/31
8/28/31	2	EB	9/14/31
9/2/31	3	VB	9/5/31
9/2/31	3	EB	9/5/31
9/2/31	3	VB	9/5/31
9/2/31	3	EB	9/5/31
9/2/31	3	VB	9/5/31
9/2/31	3	EB	9/5/31
9/2/31	3	VB	9/5/31
9/2/31	3	EB	9/5/31

The organisms from subplants could be grown under strict anerobiosis on the surface of a solid culture medium prepared by adding eight parts of VB medium to ten parts of 2 per cent veal infusion agar, containing 0.4 per cent NaH_2PO_4, having a P_H of 7.6. This medium in Petri plates, or slanted in test tubes, yielded moderate surface growth after one week or ten days.

After two or more weeks of incubation the growth became more profuse. Successful secondary cultivation was accomplished in this manner by the use of the Olitsky-Boëz apparatus[33] equipped with a palladinized asbestos catalyzer.

Subplants from positive cultures were made on aerobic blood agar plates and ordinary agar slants and in brain-heart infusion (Bacto) incubated aerobically and anerobically under sterile liquid albolene. No growth could be detected macroscopically or microscopically after one month.

To summarize.—Cultivation experiments were attempted with Berkefeld N filtrates of 5 per cent suspensions of brain and spinal cord from monkeys inoculated with poliomyelitic virus and developing typical experimental poliomyelitis. Seven strains of virus (all representing the original Flexner virus after propagation in series of monkeys) were studied in a special medium described here as EB and VB and containing minced sheep brain. Positive cultures were obtained under anerobic conditions from such virus filtrates and subplants from all positive tubes have yielded successful growth in series. Up to the present time these cultures are in the nineteenth subplant and the organisms in the original tubes have remained viable to date after an incubation period of more than twenty-two weeks at 37.5° to 38.0° C.*

Successful cultivation on solid medium has been accomplished under strictly of subplants anerobic conditions by means of the Olitsky-Boëz apparatus. The culture medium for this purpose was prepared by addition of VB sheep brain medium to buffered veal infusion agar, free from salt and peptone, and adjusted to P_H 7.6.

MORPHOLOGY

In film preparations made from inoculated culture medium, and stained by the method of Wright, the organisms were clearly defined notwithstanding the presence of microscopic bits of tissue, cellular detritus, and occasionally variable amounts of granular deposit. The Giemsa stain was found unsuitable owing to the lack of contrast in the field and a tendency toward overstaining. With the Gram technic the organisms could not be demonstrated in the early subplants although they were present in duplicate specimens prepared with the Wright stain. In older cultures and later subplants the bodies could be seen with the Gram stain and were weakly gram-positive. Hematoxylin-eosin was also found satisfactory. Under a magnification of 1500 to 2000 diameters the minute organisms with Wright stain were seen as blue or violet ovoid bodies occurring singly, in pairs, or irregular clusters, very rarely in short chains, and in densely packed masses. They varied in size from the lowest limit of visibility to 0.05 or 0.1 to 0.2 micron. Enormous numbers were seen in particles of brain tissue for which the organisms exhibited a marked predilection. They appeared on closer inspection to have a sheath-like surrounding envelop that favored the formation of masses or clumps (Figs. 2-4).

In the more remote subplants the minute bodies were larger and infrequently associated with extremely large globular or coccoid forms ranging in size from 2 to 3 or more microns in diameter. These were usually pink or reddish in color and unevenly stained, sometimes resembling the low power picture of polymorphonuclear leucocytes. Among these were also found vacuolated struc-

*32°-34° C. has proved more satisfactory.

tures having the appearance of an empty shell, and reminiscent of the "Pettenkoferiaformen" described by Kühn.[36] The large bodies were seen frequently in the first few generations derived from the primary culture, particularly when subplants were made into EB medium. Frequently, also, these forms appeared in cultures in which the small organisms had undergone disintegration and the culture appeared to be growing poorly. The minute bodies and small forms in gen-

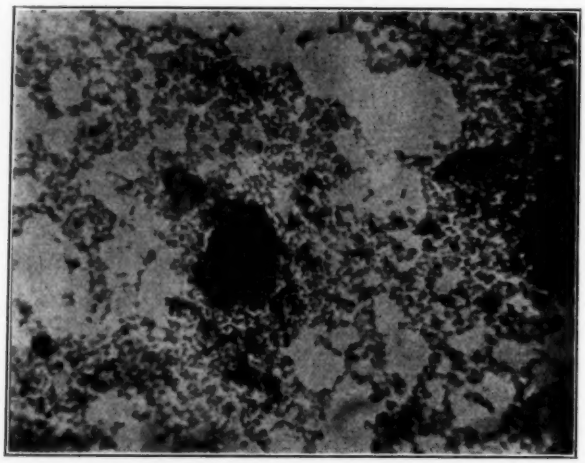

Fig. 2.—Film preparation virus culture, fifth subplant, ten days' growth, showing organisms in VB brain tissue medium. The dark areas represent dead nuclear material in the nerve tissue. (Wright stain ×1500.)

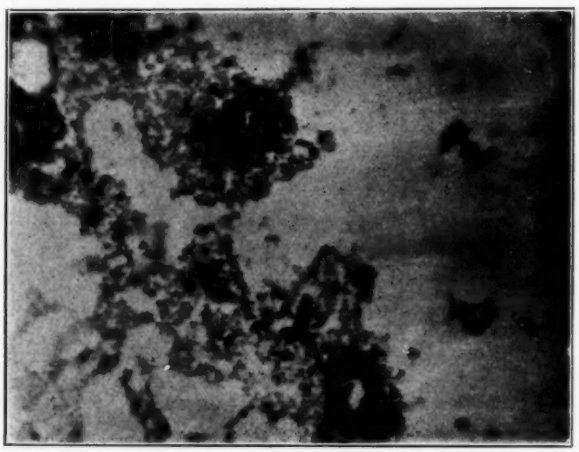

Fig. 3.—Film preparation of virus culture, showing minute bodies and masses in relation to nuclear structures in the brain tissue. (Wright stain ×1500.) (VB medium.)

eral predominated in VB culture medium, and in the later generations as the large organisms became rare, the small bodies appeared in virtually pure culture. However, a subplant from tubes containing one type exclusively might yield the other or a mixture of the two (Figs. 5-7). In a number of subplants the minute ovoid bodies appeared elongated and the surrounding sheath-like structure gave the single or grouped organisms the appearance of fusiform bacteria.

Morphology in Culture Filtrates.—Berkefeld N filtrates from cultures con-

taining the typical small and associated larger forms yielded positive cultures in VB medium after eight to thirty days' incubation. Film preparations revealed clusters of minute organisms and slightly elongated sheath-like forms stained blue. Large forms were not found. Filtrates from cultures containing only the larger globular bodies (as observed in several film preparations) were negative for growth after four weeks of incubation. Typical small forms developed in the fil-

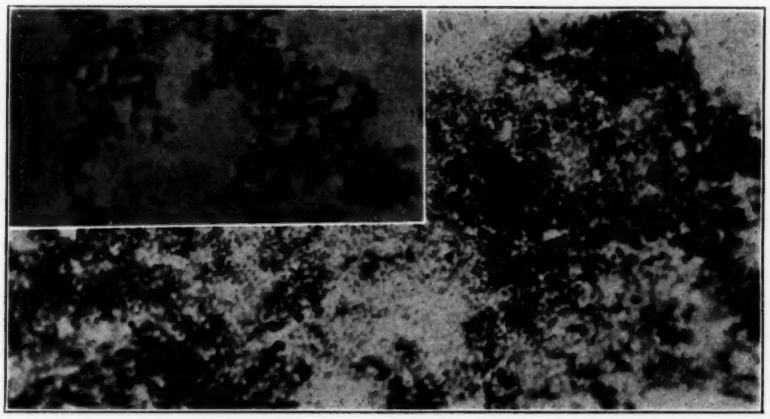

Fig. 4.—Film preparation of virus culture, showing zoogleal masses and sheath-like structure of bodies. A few large bodies are seen in the field. (Wright stain ×1500.) (EB medium.)

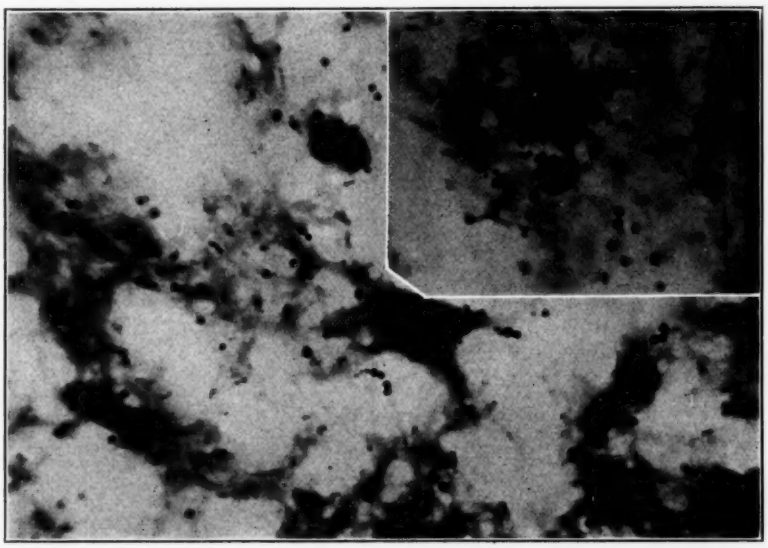

Fig. 5.—Film preparation of virus culture, sixth subplant, showing relative size of minute bodies and large globular forms in comparison with nuclear structure in brain tissue. (Wright stain ×1500.)

trates in six trials with cultures that originally contained only the typical minute bodies.

Comment.—From these preliminary filtration experiments certain permissible deductions might be made. These appear to bear some relation to the microscopic appearance observed in film preparations of cultures taken at different times. A given culture contains organisms that are optically invisible and these

develop into visible forms. For the moment the various stages of growth and possible life cycle may be disregarded. It is important to note that the mere recultivation of identical organisms from a Berkefeld filtrate of a culture need not imply the actual passage of the original bodies, however small, through the filter. A rea-

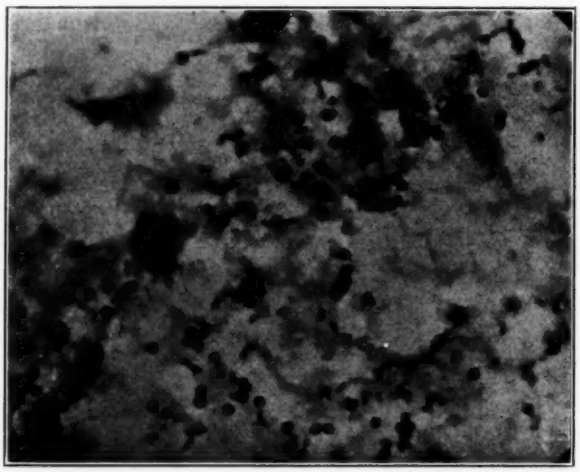

Fig. 6.—Film preparation of virus culture, sixth subplant, showing preponderance of large forms developing in the brain tissue. (Wright stain ×1500.)

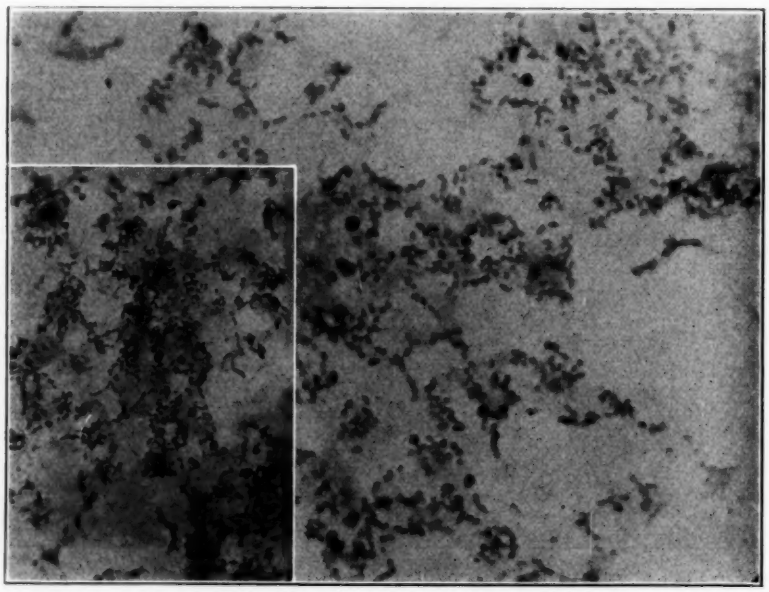

Fig. 7.—Film preparation of virus culture, sixth subplant, showing proliferation of organisms in the matrix of brain tissue. (Wright stain × 1500.)

sonable interpretation of the experiment suggests that ultramicroscopic invisible forms that are present in the filtrate may develop later into visible bodies in the culture medium. This inference cannot be accepted as a definite hypothesis without more elaboration of these experiments. In the few instances studied, no microorganisms could be demonstrated in stained film preparations made at various

times from Berkefeld N filtrates of cultures. These filtrates were subjected to prolonged centrifugalization at the highest possible speed and the stained material from the tip of a Hopkins centrifuge tube was examined microscropically under a magnification of 2000 to 3000 diameters.

To Summarize.—The typical organism isolated from Berkefeld N filtrates of poliomyelitic brain and spinal cord tissue was a very minute dot-like or ovoid body ranging in size between 0.05 to 0.1 or 0.2 micron. It appeared to have a sheath-like structure tending to cause the bodies to adhere in clumps or irregular masses. At times they occurred in small clusters, or singly or in pairs and rarely in very short chains. With the Wright stain the organisms stained blue or deep violet, sometimes reddish violet depending upon the P_H of the culture medium at the time. The reaction to Gram's stain was weakly positive in older cultures and in late generations. In primary culture and in the early subplants the bodies could not be demonstrated by the Gram method. They were clearly seen with a magnification of 1500 to 2000 diameters and appeared to have multiplied abundantly in the bits of brain tissue that constituted the culture medium. After several weeks of incubation at 37.5° to 38° C. the organisms became somewhat enlarged and stained irregularly. These cultures containing the small forms were "filterable" through Berkefeld N filters just as were the original typical minute bodies. Filtration experiments with the cultures and the recovery of the original organisms from the Berkefeld filtrate suggested the passage of temporarily invisible forms capable of developing later into visible ones in the culture medium. The actual passage of the original minute organism through a filter was not implied. The possibility of harmless "contaminants" being present in the company of typical bodies was also recognized. These associated organisms may exhibit remarkable pleomorphism and bizarre stages of growth. The deduction may not be made without further elaboration of the study that the different forms seen in any film preparation are necessarily related to a life cycle. Such an hypothesis, however tempting, might be hazardous at this time.

INFECTIVE POWER OF CULTURES IN MONKEYS

In order to establish the possibility of causal relationship of the organism to poliomyelitis, attempts were made to induce experimental infection in monkeys. For this purpose cultures were used that had been isolated from Berkefeld N filtrates of brain and cord of a monkey experimentally infected with a known virus. This strain of virus, injected intracerebrally in 0.4 to 0.5 c.c. amounts of a Berkefeld N filtrate of a 5 per cent suspension of glycerolated brain and cord produced typical poliomyelitis in seven to eight days. Smaller doses were infective only rarely or not at all in numerous attempts to arrive at an approximate minimal dose in medium-sized monkeys that were at our disposal. The cultures used in the experiments to be described were obtained from a monkey with the following history:

Experiment 1.—Macacus rhesus inoculated intracerebrally on February 28, 1931, with 0.5 c.c. of a Berkefeld N filtrate of a 5 per cent suspension of glycerolated virus recently obtained from a monkey in the paralytic stage of poliomyelitis (courtesy of Dr. Simon Flexner, Rockefeller Institute). March 6, excitement and lessened activity were observed. March 7, body tremor, facial paralysis, and paralysis of right arm were noted. March 9, ataxia, weakness of both legs and left arm were observed. The animal was prostrate and unable to move its legs and arms later in the day. Etherized. The lesions in gross and histopathologic sections were

characteristic of experimental poliomyelitis. Infected nervous tissues of this animal were preserved in sterile 50 per cent glycerol and stored in the ice chest at 4° C. for five and one-half months. At this time Berkefeld N filtrates of a 5 per cent suspension of the brain and cord were inoculated into the special culture medium from which were derived the cultures that have been described.

Monkeys were inoculated with materials from the cultures in their third, fourth, seventh, eighth, tenth, eleventh, and thirteenth subplants. It should be noted that the fourth transfer already represented a dilution of about 2×10^{-9} of the original inoculum that had been used for primary cultivation in amounts of 0.1 c.c. of a Berkefeld N filtrate. This amount of virus by itself was considerably below the known infective dose, aside from its serial dilution in the subplants.

METHOD OF PREPARATION AND INOCULATION OF CULTURE MATERIAL

The material used for inoculation of monkeys consisted of the supernatant contents of several culture tubes incubated eight to twelve days, well shaken and mixed when removed from the incubator, and centrifuged for two to three minutes at 125-150 R.P.M. In a few instances four, six, and twenty-five day old cultures were used. As a general rule subplants were prepared from tubes that had been incubated for two to three weeks, occasionally from some six to seven weeks old, and in two instances from sixty-five and one hundred day old cultures. Experience showed that cultures less than ten days old were best suited for inducing an infection. In one series of experiments (eleventh subplant) sedimented organisms, washed 3 or 4 times with salt solution, were used. All materials were examined microscopically in film preparations before injection into monkeys and only satisfactory specimens free from ordinary contaminating bacteria were employed. The culture used in the inoculation experiments will be designated as Strain 3.

Certain points that may modify the outcome of inoculations should be emphasized here. It is especially important to secure an actively growing culture preferably not more than nine days old. This can be ascertained from film preparations made at the stated time. The organism has certain peculiarities and fastidious requirements. It will be noted that multiplication takes place in the bits of brain tissue constituting the major part of the culture medium. Hence it is essential to secure material from cultures having abundant organisms in this dead yet chemically and biologically suitable tissue substrate. The amount of material used for inoculation is an important factor, notably in animals such as the monkey that is only relatively susceptible to poliomyelitis. To cause a primary infection in the monkey will require a considerable amount of culture material. This amount is not in any sense extraordinary considering the type of material inoculated and the slow growth of the organism. Once the disease has been induced, however, serial passage can be effected in the monkey by means of small amounts of Berkefeld filtrates or of suspensions prepared from the nervous tissues of the infected animals.

The following method was adopted: The contents of a number of culture tubes, generally 4 to 6 in number, were transferred under strict aseptic precautions to centrifuge tubes having a capacity of 50 c.c. In order to facilitate the passage of tissue particles, a 10 c.c. pipette was used with the narrow outlet broken off to increase the diameter. Suction was applied by means of a large rubber bulb at-

tached to the mouthpiece. After light centrifugation at the speed indicated, all the material overlying the gross sediment was carefully removed. This material passed without difficulty through the 21 and 22 gauge needles used in the course of inoculations, and could be injected safely into the blood stream either by way of a suitable vein or more expeditiously by direct injection into the heart.

Route of Inoculations.—Severel different routes through which infection might be accomplished were tried. The primary object was to induce localization of the organism in the brain without the theoretical possibility of virus "contamination." In order to favor penetration of the virus culture material, certain accessory procedures were used. The method of choice was to bring the organisms less directly and more slowly to the brain by way of the blood stream. To this end an injection of 10 per cent starch solution or physiologic salt solution was given into the brain and the culture into the blood stream and peritoneal cavity. In a few instances serial passages were aided by a preliminary injection into the spinal canal of normal monkey serum or of salt solution. When direct inoculation of organisms into the brain was attempted, this method was always combined with the intravenous and intraperitoneal injections or with the intravenous alone. The inoculation into the cerebrum of cultures, starch solution or salt solution served as an irritant to the brain tissues. Although we have had successful results with a single injection of a small amount of culture into the brain alone, it is again stressed here that virulence and other unknown factors may modify the end-results.

Flexner[37] has emphasized the fact that the negative infecting power of massive intravenous doses of a virus can be altered through an aseptic meningeal irritation induced by the intraspinal or intracerebral injection of a normal serum or of salt solution. Similarly, it has been shown for Borna virus[38] that the infection can be produced experimentally according to the same principle. Sawyer and Lloyd[39] studying yellow fever virus were able to infect mice by the intraperitoneal route following intracerebral injection of a 2 per cent solution of starch.

In a preliminary experiment direct inoculation was made into the cerebral hemisphere of two monkeys and into the peritoneal cavity of a third animal following an intracerebral injection of physiologic salt solution. The cultures used were in the third and fourth subplants and these transfers represented a dilution of the original material cultivated of approximately 2×10^{-7} and 2×10^{-9}, respectively. Relatively small amounts of culture were injected in order to test the virulence of the organism. Apart from a variable degree of weakness of certain groups of muscles and clinical symptoms suggestive of experimental poliomyelitis, no typical paralyses were observed in this first series.

Experiment 2.—Macacus rhesus 17: September 5, 1931, 1 c.c. of supernatant material from one positive culture Strain 3, incubated four days in the third subplant in VB medium, was inoculated into the cerebral hemisphere. The animal remained normal until September 13, on the eighth day following inoculation, when tremors of the head and body, slowness in movement, weakness of extremities, loss of appetite, and fatigability were noted. This condition improved gradually during the succeeding three days. September 17, appeared normal.

Macacus rhesus 27: September 11, 1931, 1 c.c. of supernatant material from Strain 3 incubated nine days in the third subplant in VB medium was inoculated into the left cerebral hemisphere. September 30, no symptoms having developed at this time, a second inoculation was made into the hemisphere with 1 c.c. of a culture incubated twenty-one days, in the fourth

subplant. The animal remained normal until October 6, when it became quiet and could not chew its food. There was a suggestive right facial paralysis and weakness of the buccinator muscles on the same side. October 7, right facial paralysis was more advanced; food dropped from mouth and the animal was unable to empty pouch; weakness of arms and legs and back. Improvement occurred during the next four days. October 12, appeared normal.

Macacus rhesus 16: October 1, 1931, 3 c.c. physiologic salt solution were injected into the left cerebral hemisphere and 6 c.c. of a culture incubated twenty-five days in the fourth subplant were inoculated into the peritoneal cavity. October 21, quiet, slow in movement, loss of appetite. October 22, weakness of deltoid muscles and extremities, especially on right side, body tremor, and tremor of arms and legs were observed. Gradual improvement until October 25, when animal appeared quite normal.

In the next series of experiments somewhat larger amounts of culture were inoculated. These were by no means massive doses but represented pooled material from three to six culture tubes. It will be recalled that the enormous numbers of "globoid bodies" inoculated by Olitsky represented material from "cultures" contained in thirty or more tubes.

Experiment 3.—Macacus rhesus A: October 1, 1931, pooled supernatant material from 3 positive cultures incubated twenty-five days in the fourth transplant was prepared from Strain 3. Two c.c. physiologic salt solution were injected into the right cerebral hemisphere, and 6 c.c. of the culture were injected into the peritoneal cavity. October 23, 0.5 c.c. culture was injected into the left hemisphere and 1 c.c. into the peritoneal cavity. October 26, head and body tremor; ataxic gait; general weakness; completely paralyzed and prostrate later in the day. Died.

Autopsy: Meninges were slightly congested. A small area of softening was disclosed near the site of the first inoculation. No hemorrhage was noted. The cord in the area of the cervical and dorsal enlargements showed injection and petechial hemorrhage in the region of the anterior horn cells. Smears from these areas and ordinary culture tests from the brain and cord were negative. Microscopic Examination: In the forebrain only a slight mononuclear cellular reaction was observed. Proliferation of the glial cells was very slight to moderate in extent. Nerve cell degeneration was not pronounced. The medulla showed some round cell infiltration and moderate perivascular infiltration. Degeneration of nerve cells was not marked. In the cord, particularly the cervical and dorsal enlargements, were found moderate lymphocytic and leucocytic infiltrations, and slight destruction of the anterior horn cells. Neurophagocytosis was not very definite.

A 5 per cent saline Berkefeld N filtrate was prepared from pieces of the brain and cord and 1 c.c. of this material was injected intracerebrally into a monkey (No. 43). This animal developed typical experimental poliomyelitis on the sixth day with complete flaccid paralysis on the seventh day (Fig. 8).

Cultivation experiments in VB medium were carried out with Berkefeld N filtrate prepared from 10 per cent suspensions of the brain and cord of Monkey A. These tissues had been preserved in 50 per cent glycerol for ten weeks. The typical small organisms were found in the tubes of culture medium after an incubation period of eighteen to twenty-one days.

Experiment 4.—Macacus rhesus B: October 21, 1931, 2 c.c. of a 10 per cent starch solution were injected into the hemisphere and a culture prepared from Strain 3 in the fourth subplant incubated six days was inoculated as follows: 6 c.c. were injected into the peritoneal cavity and 8 c.c. intravenously. *This subplant was derived from a culture in the third generation that had been kept at incubator temperature forty-two days.* October 31, well marked head and body tremor; excitability; staccato cry. November 1, paralysis of extremities on right side; left arm and left leg very weak; ataxic gait; fur ruffled; high excitability (Fig. 9). November 2, complete paralysis, prostrate; moribund. Etherized.

Autopsy: Meninges and superficial cortical vessels were moderately injected. On cut

surface the brain and cord showed edema and areas of petechial hemorrhage in the gray matter and particularly in the region of the anterior horn cells. These changes were more marked in the cervical and dorsal enlargements of the spinal cord. Ordinary cultures and film preparations from the surfaces of the fresh nervous tissue were negative for microorganisms.

Sections from the cerebellar cortex showed slight venous congestion in the pia. The arachnoidal endothelium appeared normal. There was a small hemorrhage about which were a few lymphocytes, and scattered sparsely in the pia were mononuclear cells, mostly of undifferentiated type, occasionally showing the morphology of plasma cells. The undifferentiated type of cell possessed a relatively smaller and more deeply staining nucleus than the plasma

Fig. 8.—*Monkey 43.* Complete flaccid paralysis seven days after inoculation with poliomyelitic nervous tissue from monkey A infected with culture Strain 3.

Fig. 9.—*Monkey 39.* Complete paralysis upper extremities, neck and back muscles and beginning paralysis of lower extremities. Tenth day after inoculation with culture in fourth subplant in VB medium, six day growth.

cell, and the cytoplasm was frequently more abundant, less sharply outlined, and much less basophilic than the plasma cell. The cortex proper showed normal lamination. The nerve cells appeared negative. No inflammatory reaction or degenerative alteration was seen.

Sections from the basal ganglia showed a bit of ependymal lining to be normal. In the tissue generally, there were a few scattered venules showing distinct perivascular infiltration with lymphocytes and plasma cells.

Sections from the cerebellum showed a slight scattered infiltration with plasma cells and mononuclear cells. The cerebellar tissues, generally, appeared negative.

Sections from the spinal cord showed in the pia rather slight inflammatory reaction, except in the extension of the pia into the anterior fissure, where a variable amount of lymphocytic

and plasma cell infiltration occurred in the different sections. The general architecture of the anterior horns, and to a lesser extent posteriorly, was disturbed by diminution in number of the anterior horn cells. Some were absent, others represented by fragments of nerve cells, and some showed infiltration about them. Neurophagocytosis was quite prominent.

There were also tiny patches of cellular infiltration independent of obvious degenerating cells. A few petechial hemorrhages were seen. The cellular exudate about degenerating areas

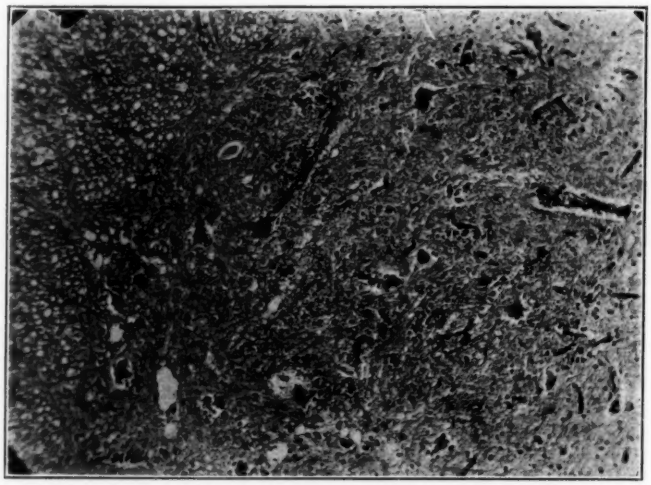

Fig. 10.—*Monkey 39.* Section spinal cord, showing extensive destruction of anterior horn cells and perivascular infiltration; vascular hemorrhages and congestion and diffuse round cell infiltration; focal accumulations of leucocytes and moderate neurophagocytosis.

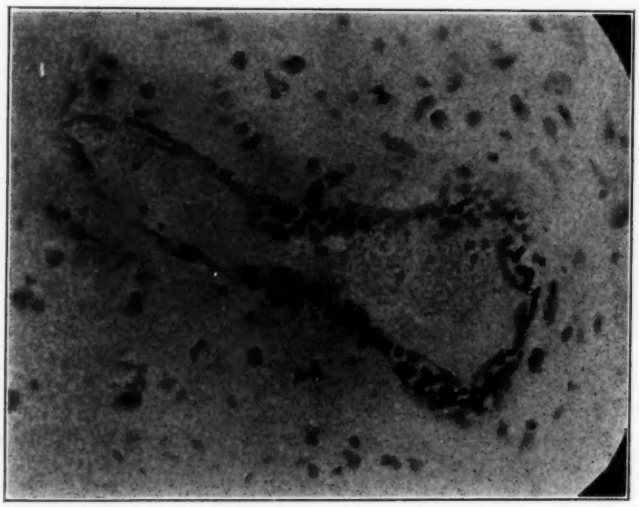

Fig. 11.—*Monkey 39.* Section brain, Nissl stain, showing round cell perivascular infiltration and nerve cell necrosis (×400).

consisted of undifferentiated mononuclear cells; occasionally slight perivascular infiltration occurred, and there was apparently some beginning hypertrophy of the glial elements in this region (Figs. 10-13).

The next experiment was an attempt to transmit experimental poliomyelitis to normal monkeys from those that had been infected by means of the culture.

Three monkeys were inoculated with suspensions of Berkefeld N filtrate pre-

pared from the brain and cord of Monkey B in Experiment 4. All three control animals developed typical poliomyelitis, two within eight days and one on the tenth day. These results are given in Experiment 5.

Experiment 5.—Macacus rhesus 36: November 9, 1931, 2 c.c. normal monkey serum were injected intraspinally. November 10, 1 c.c. of a 10 per cent suspension prepared from the

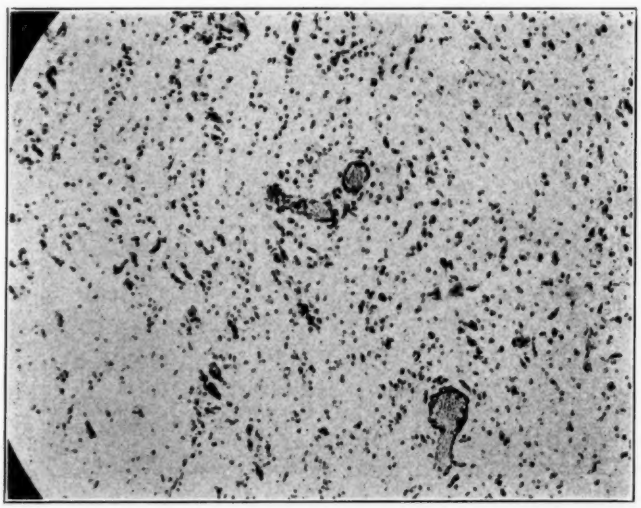

Fig. 12.—*Monkey 39.* Section brain, Nissl stain, showing round cell perivascular infiltration and nerve cell necrosis (×100).

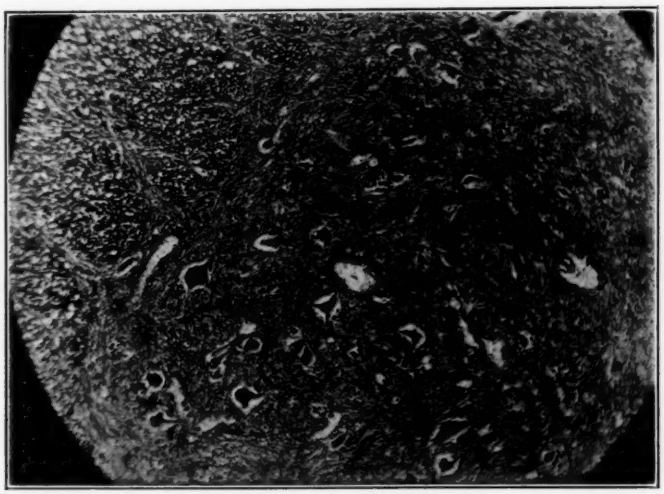

Fig. 13.—*Monkey 29.* Normal monkey. Section spinal cord. Note number normal intact nerve cells and absence of inflammatory changes in comparison with Fig. 10.

brain and cord of Monkey B was inoculated into the right cerebral hemisphere. November 16, excitement, tires easily, fur ruffled. November 18, complete paralysis of the extremities and back muscles; prostrate. November 22, died. The gross and histopathologic examinations revealed characteristic pronounced lesions of experimental poliomyelitis. (Figs. 14-16).

Macacus rhesus 37: November 9, 1931, 1 c.c. of a 10 per cent suspension prepared from the brain and cord of Monkey B was inoculated into the cerebral hemisphere. November 16, weakness of extremities, ataxic gait, body tremor, excitability, head tremor, staccato cry, right facial paralysis. November 17, complete paralysis; prostrate; unable to move arms and legs.

Etherized. The gross and histopathologic examinations revealed characteristic well marked lesions of experimental poliomyelitis.

Macacus rhesus 38: November 9, 1931, 1 c.c. of a 10 per cent saline Berkefeld N filtrate prepared from the brain and cord of Monkey B was inoculated into the cerebral hemisphere. November 19, head and body tremor, excitability, weakness of extremities and back muscles, ataxia. November 20, complete paralysis of extremities; prostrate; unable to move arms and

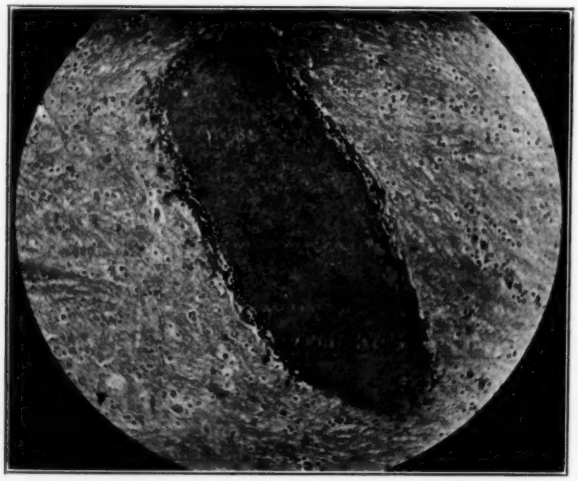

Fig. 14.—*Monkey 36.* Complete paralysis eighth day, died twelfth day; inoculated with nervous tissue from monkey 39, representing second virus culture passage. Section of brain, showing perivascular infiltration of cerebral blood vessel and diffuse round cell infiltration.

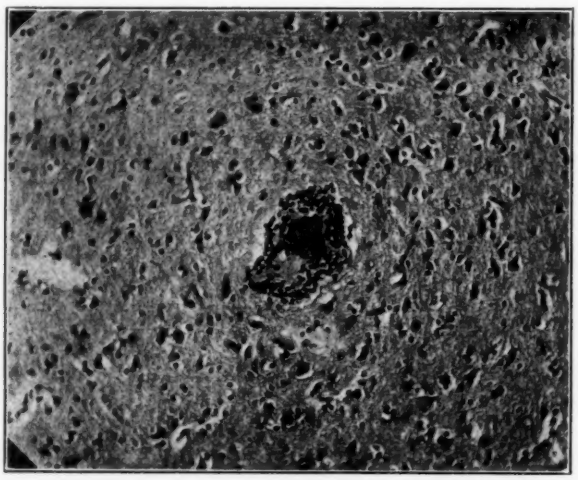

Fig. 15.—*Monkey 36.* Section of brain, showing round cell infiltration of smaller blood vessel and diffuse inflammatory reaction.

legs. Etherized. Gross and histopathologic examinations revealed typical and pronounced lesions of experimental poliomyelitis (Fig. 17).

Cultivation experiments in VB medium were made with 10 per cent saline Berkefeld N filtrates and suspensions prepared from the brain and cord of Monkey B and of Monkeys 37 and 38. In the first and successive transfers, minute bodies resembling the typical original cultures, were isolated. The nervous tissues from these animals had been preserved in 50 per cent glycerol and stored in

the ice chest at 4° C. for periods ranging from three hours to four days, and up to and including seven weeks. Tests for sterility in ordinary culture medium were uniformly negative after three to four weeks of incubation.

Two normal monkeys were inoculated with one of these cultures recultivated from the brain and cord of Monkey 38. One animal developed symptoms and

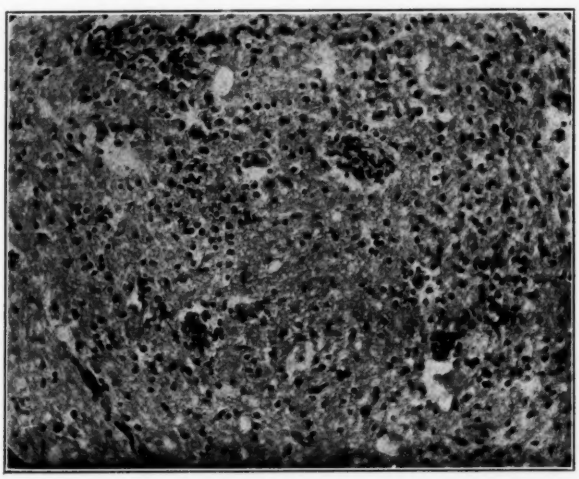

Fig. 16.—*Monkey 36.* Section of spinal cord, showing focal accumulation of round cells with marked destruction of nerve cells, diffuse round cell infiltration and neurophagocytosis.

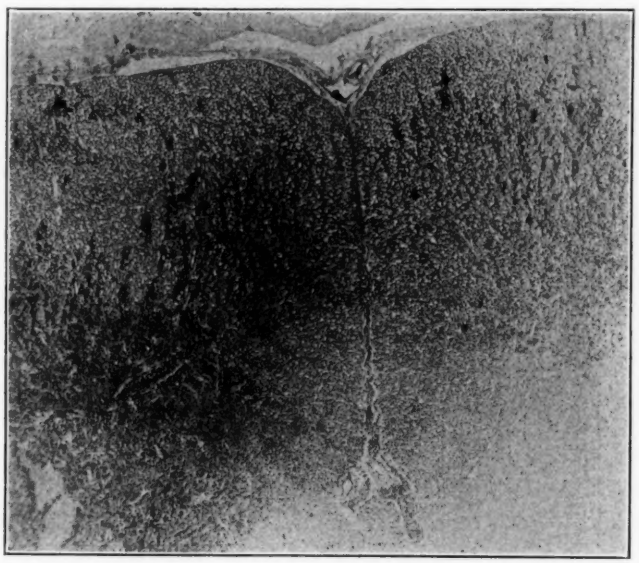

Fig. 17.—*Monkey 38.* Complete paralysis, eleventh day; inoculated with Berkefeld filtrate nervous tissue from monkey 39. Section of spinal cord, showing vascular lesion extending from meninges inward along anterior fissure; diffuse infiltration of blood vessel.

paralysis on the twelfth day following inoculation with complete recovery. The second animal showed symptoms on the fifteenth day, became prostrate and died on the sixteenth day following inoculation. The culture used was prepared from a pooled seven day growth of organisms in V B medium and representing the second subplant. The results are summarized in the following experiment.

Experiment 6.—Macacus rhesus 59: December 12, 1931, 2 c.c. of a 10 per cent starch solution were injected into the right cerebral hemisphere, 5 c.c. of pooled culture material intravenously, and 5 c.c. into the peritoneal cavity. December 19, animal was normal. December 24, tires easily, left deltoid muscle weak. December 28, excitability marked, left deltoid defi-

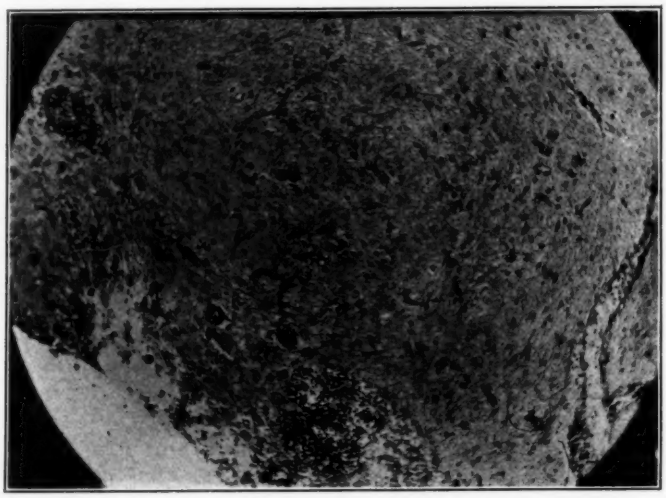

Fig. 18.—*Monkey 53.* Complete paralysis eighth day, died on ninth day; inoculated with suspension nervous tissue from monkey 38. Section spinal cord, showing diffuse infiltration, focal accumulation of round cells and vascular lesion extending inward from meninges. (Third virus culture passage.)

Fig. 19.—*Monkey 56.* Complete paralysis, bulbo spinal infection and death on fourth day; inoculated with Berkefeld filtrate nervous tissue from monkey 38. Left facial paralysis on fourth day after inoculation. (Third virus culture passage.)

nitely paralyzed. No other involvement was observed. January 4, 1932, excitability decreased, left deltoid still weak. January 22, animal appeared to be quite normal.

Macacus rhesus 61: December 11, 1931, 2 c.c. normal monkey serum were injected intraspinally. December 12, 2 c.c. culture were injected into the right cerebral hemisphere, 5 c.c.

intravenously and 5 c.c. into the peritoneal cavity. December 24, animal was normal. December 27, marked excitability. December 28, arms and legs very weak; weakness of back and neck muscles. Later in the day the animal became completely paralyzed and prostrate. It was unable to move arms or legs. Death from respiratory failure. The gross and histopathologic examinations revealed characteristic lesions of experimental poliomyelitis in the spinal cord and brain.

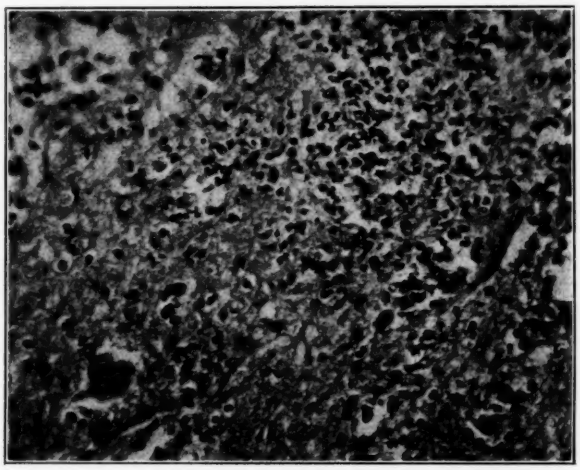

Fig. 20.—*Monkey 56.* Section from spinal cord, showing large focal accumulation of round cells and leucocytes and absence of nerve cells.

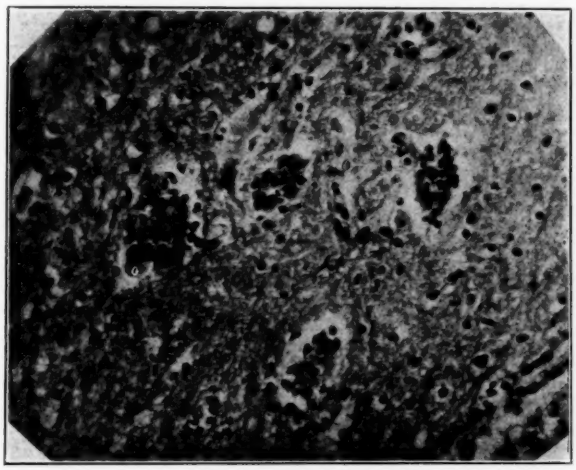

Fig. 21.—*Monkey 56.* Section from spinal cord, cervical enlargement, showing pronounced neurophagocytosis, diffuse round cell infiltration and destruction of anterior horn cells.

From these experiments it may be deduced that experimental poliomyelitis is transmissible with a culture recovered from culture-infected monkeys.

In the following experiment an attempt was made to induce experimental poliomyelitis in normal monkeys with materials prepared from the nervous tissues of animals representing a second successful passage of the culture Strain 3.

Four monkeys were inoculated with suspensions or Berkefeld N filtrates of materials prepared from the brain and cord of Monkeys 37 and 38 in Experiment 5. The results are summarized in Experiment 7.

Experiment 7.—Macacus rhesus 52: November 18, 1931, 1 c.c. of a 10 per cent saline Berkefeld N filtrate prepared from the brain and cord (one day in 50 per cent glycerol at 4° C.) of Monkey 37 was inoculated into the right cerebral hemisphere. November 26, excitability, ataxia, complete paralysis; prostrate; unable to move arms and legs.

Macacus rhesus 53: November 18, 1931, 1 c.c. of a 10 per cent suspension prepared from the brain and cord of Monkey 37 was inoculated into the right cerebral hemisphere. November 24, excitability, ruffled fur, staccato cry, tires easily. November 25, head and body tremor; left facial paralysis; left arm paralyzed. November 26, prostrate; unable to move arms and legs. November 27, died. The gross and histopathologic examinations revealed well marked lesions characteristic of experimental poliomyelitis (Fig. 18).

Macacus rhesus 55: November 20, 1931, 1 c.c. of a 10 per cent suspension prepared from the brain and cord (three hours in 50 per cent glycerol at 4° C.) of Monkey 38 was inoculated into the right cerebral hemisphere. November 24, excitability, ruffled fur. November 25, left facial paralysis, left arm paralyzed, head and body tremor; right arm and legs and back muscles very weak. November 26, complete paralysis; prostrate. December 22, died. Histopathologic examination revealed typical lesions of experimental poliomyelitis.

Macacus rhesus 56: November 20, 1931, 1 c.c. of a 10 per cent saline Berkefeld N filtrate prepared from the brain and cord of Monkey 38 was inoculated into the right cerebral hemisphere. November 24, head and body tremor, left facial paralysis; beginning paralysis of upper extremities; marked general weakness. Nine hours later, marked ataxia, more advanced paralysis of arms, and weakness of muscles of neck and back (Fig. 19). Later in evening, total paralysis; picture of rapidly progressing bulbar type of poliomyelitis; respiratory failure and death. Gross and histopathologic examinations revealed profound tissue changes characteristic of experimental poliomyelitis (Figs. 20 and 21).

Cultivation experiments in VB medium were made with Berkefeld N filtrates and suspensions prepared from the brain and cord of Monkeys 53 and 56. An organism resembling the original Strain 3 was isolated and grown in serial subplants.

From the preceding experiments one observes that the inoculation of suspensions or Berkefeld filtrates prepared from nervous tissue taken from monkeys experimentally infected with poliomyelitis by means of cultures induces typical clinical and pathologic effects. These effects are transmissible in series and, as shown in Experiment 7, appear to be augmented as a result of successive passage of the culture material through a series of monkeys. Notable in this respect is the definite shortening of the period of incubation. The recaptured "virus" appears to retain its original properties of filterability and pathogenicity. The results of some of the earlier experiments in monkeys and the transmission of poliomyelitis by passage through a series of animals are summarized in Table II.

The infective power of a culture in the eighth subplant was studied in the following experiment. The material represented a dilution of the original inoculum cultivated of approximately 2×10^{-17}.

Experiment 8.—Macacus rhesus 2636: November 25, 1931, the supernatant fluid from one culture was ground in a mortar with sterile quartz sand, and centrifuged for three minutes at 150 r.p.m. One cubic centimeter of the resultant turbid supernatant material was injected into the cerebral hemisphere. December 2, excitement, tremor and ataxia were noted; both arms almost totally paralyzed; prostrate. General weakness progresses and the animal was etherized.

Autopsy.—Visible lesions were seen in the cervical and lumbar cord; viscera were normal. Smears from the brain surface and base and the site of inoculation were negative. Cultures in ordinary solid and fluid media were negative. Histopathologic examination revealed typical lesions of experimental poliomyelitis in the cord and brain.

TABLE II

SUMMARY OF EARLY EXPERIMENTS IN WHICH AN ORGANISM ISOLATED FROM POLIOMYELITIS VIRUS FILTRATE WAS ADAPTED TO A CULTURE MEDIUM CONSISTING OF MINCED SHEEP BRAIN TISSUE SUSPENDED IN PEPTONE-FREE, GLUCOSE-VEAL INFUSION

Poliomyelitis Virus Filtrate, First Culture (3)

C 2

C 3

C 4

(icer and iper) — M. rhesus 5 — (prostrate nineteenth day)

(icer susp. 10% brain and cord) — M. rhesus 43 — (complete paralysis seventh day)

Poliomyelitis Virus Filtrate Culture, Fourth Generation

(icer starch sol., iven and iper culture) M. rhesus 39 (prostrate eleventh day; died twelfth day; culture recovered)

M. rhesus 36
icer susp. 10 % brain and cord glycerolated 7 days; prostrate eighth day; died twelfth day

M. rhesus 37
icer susp. 10 % brain and cord glycerolated 6 days; prostrate eighth day; culture recovered

- M. 52
  icer Berkefeld filtrate susp. 10 % brain and cord; complete paralysis and prostrate eighth day
- M. 53
  icer susp. 10 % brain and cord; complete paralysis seventh day; prostrate eighth day; died ninth day; culture recovered

M. rhesus 38
icer Berkefeld filtrate susp. 10 % brain and cord glycerolated 6 days; prostrate eleventh day; culture recovered

- M. rhesus 55
  icer susp. 10 % brain and cord; complete paralysis and prostrate fifth day; died thirty-second day
- M. rhesus 56
  icer Berkefeld filtrate susp. 10 % brain and cord; prostrate and died fourth day; culture recovered

Macacus rhesus 2586: December 3, 1931, 1 c.c. of a 10 per cent suspension of material prepared from the brain, cervical and lumbar cord of Monkey 2636 was injected into the cerebral hemisphere. December 10, slight tremor, ptosis and ataxia were observed. December 11, tremor and ataxia were more marked and both arms wer totally paralyzed. December 12, animal was prostrate and unable to move arms or legs. Etherized. Autopsy revealed visible gross lesions in the spinal cord. The viscera were normal. Cultures prepared with material from the nervous tissues were negative for organisms in ordinary culture media. Histopathologic examination revealed lesions in the brain and cord typical of experimental poliomyelitis.

In the next experiment virus cultures in the eleventh subplant were inoculated into a series of monkeys. These transfers represented a dilution of the original material cultivated of approximately 2×10^{-25}. Four monkeys were inoculated and three developed the typical clinical syndrome and paralysis of experimental poliomyelitis within seven days, and the fourth animal on the tenth day. Of these, two received sedimented cultures washed three times with normal physiologic salt solution. The supernatant pooled material from five culture tubes that had been previously mixed and centrifugalized lightly for two to three minutes was sedimented at high speed. The resultant sediment containing the organisms was washed three times after preceding centrifugations. This material represented a culture in the eleventh subplant incubated eight days at 37.5° to 38° C.

Experiment 9.—Macacus rhesus C: December 17, 1931, 2 c.c. of washed culture were injected into the left cerebral hemisphere, 5 c.c. intravenously, and 5 c.c. into the peritoneal cavity. December 23, both arms paralyzed, neck and back muscles weak. Ten hours later, the animal was totally paralyzed, prostrate and moribund. Etherized. The infection advanced in the manner characteristic of a bulbar type. Gross and histopathologic examinations revealed characteristic lesions of poliomyelitis in the spinal cord and brain.

Macacus rhesus D: December 17, 1931, 2 c.c. of a 10 per cent starch solution were injected into the left hemisphere, 5 c.c. of washed culture intravenously, and 5 c.c. into the peritoneal cavity. December 22, excitability, weakness of arms, slight muscle tremor, tires easily, beginning right facial paralysis. December 23, marked right facial paralysis, total paralysis of extremities, neck and back; prostrate. Etherized. The gross and histopathologic findings were typical of poliomyelitis in the brain and spinal cord.

Macacus rhesus E: December 17, 1931, 2 c.c. of a 10 per cent solution of starch into the left hemisphere, 5 c.c. of pooled culture into the peritoneal cavity, and 5 c.c. intravenously. December 23, irritability and muscle tremors were noted. December 24, right facial paralysis, marked head and body tremor, weakness of right arm and leg, paralysis of left arm and leg, weakness of back muscles, and ataxia. Prostrate six hours later.

Macacus rhesus F: December 17, 1931, 2 c.c. pooled culture into the left cerebral hemisphere, 5 c.c. into the peritoneal cavity, and 5 c.c. intravenously. December 25, normal. December 27, excitability and weakness of right deltoid and both legs were noted. December 28, legs completely paralyzed, right arm paralyzed and left deltoid weak. December 30, prostrate, and unable to move arms or legs.

A 10 per cent suspension was prepared from portions of the brain and cord of Monkey C, and of this material 0.4 c.c. was injected intracerebrally into seven monkeys. All these animals developed typical symptoms and paralyses followed by complete prostration after a period of from six to ten days. Four animals died on the seventh to the eighth day following inoculation. Gross and histopathologic examinations revealed typical and well marked lesions in the spinal cord, medulla and brain, that were characteristic of experimental poliomyelitis.

Cultivation experiments in VB medium were carried out with Berkefeld filtrates prepared from cerebral nervous tissue from Monkey C. These tissues had been preserved in 50 per cent glycerol for 14 days. Typical cultures were ob-

tained after an incubation period of 22 days in the culture medium. In all respects the organisms were identical with the original strain with which Monkey C had been inoculated.

Two monkeys were inoculated with cultures in the thirteenth subplant, representing a dilution of the original material cultivated of approximately 2×10^{-27}. Both animals developed typical symptoms and were paralyzed within one week.

Experiment 10.—Macacus rhesus 68: January 6, 1932, 1 c.c. culture from pooled supernatant material from three tubes of the thirteenth transfer was injected into the right cerebral hemisphere, 5 c.c. intravenously and 5 c.c. into the peritoneal cavity. January 12, prostrate, complete paralysis, moribund. Etherized.

Autopsy.—The site of inoculation showed nothing unusual and film preparations were negative. The cut surfaces of the spinal cord, particularly in the cervical region and the medulla, showed hemorrhagic pin point areas. Histopathologic examination of the medulla and spinal cord revealed marked perivascular infiltration, nerve cell degeneration and neurophagocytosis and focal accumulations of lymphocytes. Meningeal reaction was pronounced in the cervical and thoracic and, to a lesser degree, in the lumbar cord.

Macacus rhesus 69: January 6, 1932, 1 c.c. of culture from pooled supernatant material from three tubes of the thirteenth transfer was injected into the cerebral hemisphere, 5 c.c. into the peritoneal cavity and 5 c.c. intravenously. January 12, both legs paralyzed, left deltoid weak. January 13, prostrate; February 21, died.

Autopsy: Tuberculosis of lungs. Histopathologic examination revealed typical lesions in the medulla, cord, and to a slight extent, in the brain. The changes were regressive and not like those seen in the acute stages.

The infective power of cultures heated for one hour at 65° C. was next studied in two monkeys. One received a preliminary injection of starch solution and another an intraspinal injection of normal monkey serum.

Experiment 11.—Macacus rhesus 48: November 18, 1931, 1 c.c. of normal monkey serum was injected intraspinally and on the following day 5 c.c. of a suspension prepared from 3 pooled cultures in the fourth subplant, incubated nine days and heated for one hour at 65° C., were injected intravenously.

Macacus rhesus 49: November 19, 1931, 1 c.c. of a 10 per cent starch solution was injected into the right cerebral hemisphere and 5 c.c. of a suspension prepared from 3 pooled cultures in the fourth subplant, incubated nine days and heated for one hour at 65° C., were injected into the peritoneal cavity, and 5 c.c. of the same material intravenously.

The monkeys remained well and were discarded after one month's observation.

Materials from culture tubes in the first, second, third, and fourth subplants that contained no demonstrable organisms were also inoculated into two monkeys as illustrated in the following experiment.

Experiment 12.—Macacus rhesus 18: September 5, 1931, 1 c.c. of pooled material from four negative tubes containing no demonstrable organisms, of the first to fourth subplants of Strain 3, was inoculated into the left cerebral hemisphere.

Macacus rhesus 19: September 16, 1931, 1 c.c. of pooled material from three negative tubes containing no demonstrable organisms, of the first and second subplants of Strain 3, was inoculated into the right cerebral hemisphere. October 1, 1931, 2 c.c. physiologic salt solution were injected into the left hemisphere and 7 c.c. of material prepared from the contents of four negative tubes containing no demonstrable organisms, of the first and second subplants, were inoculated into the peritoneal cavity.

The monkeys remained well and were discarded after one month's observation.

From the preceding experiments one may observe that the inoculation in monkeys of organisms obtained from cultures of poliomyelitic tissue in VB me-

dium caused symptoms and signs and pathologic changes in the nervous system characteristic of experimental poliomyelitis. Successful experiments were accomplished with culture subplants representing a dilution ranging from approximately 2×10^{-7} and 2×10^{-9} to 2×10^{-27} of the original material cultivated, namely, 0.1 c.c. of a 5 per cent saline Berkefeld filtrate of poliomyelitic nervous tissue. In parallel experiments in which cultures heated for one hour at 65° C. were used, the inoculations in monkeys were without effect. Likewise, additional experiments with materials derived from culture tubes that contained no demonstrable organisms resulted negatively.

INFECTIVITY EXPERIMENTS IN RABBITS, GUINEA PIGS AND MICE

It is well known that ordinary streptococci, or bacteria, when injected directly into the brain or into the circulation of monkeys or smaller animals give rise to symptoms that are not characteristic of poliomyelitis. As a general rule the outcome of inoculations with streptococci is referable to the production of frank meningitis, purulent cerebral abscesses, or septicemia and death within forty-eight hours or less.

The work of Rosenow and associates[40] and others[41] has been thoroughly reinvestigated, notably by Bull,[42] Smillie,[43] Amoss and Eberson,[44] and Olitsky and Long.[45] Flexner[46] in his earliest work had already shown that the rabbit was resistant to poliomyelitic infection. There should be no question as to the complete absence of any relationship between the streptococcus described by Rosenow or others, and what is recognized as poliomyelitis in the experimental animal. An examination of such protocols, as are given in these publications, establishes forthwith the fact that in no instance was poliomyelitis induced with cultures of streptococci. The clinical picture of the disease was conspicuously absent. The short period of incubation and rapid course of infection, its outcome, and the gross pathology did not correspond in the remotest degree to poliomyelitis.

With these points in mind it was thought desirable to study in the rabbit, and smaller laboratory animals, the behavior of the organisms isolated by us. Rabbits were inoculated into the cerebral hemisphere with 0.2 to 0.4 of a cubic centimeter of material representing three or more pooled cultures grown for twelve days in the incubator. Another series of rabbits was injected intravenously with from 3 to 5 c.c. of similar materials. Guinea pigs received intraperitoneal injections of from 3 to 5 c.c. of identical cultures, and finally a group of mice was inoculated into the peritoneal cavity with from 1 to 2 c.c. of a suspension of culture. The animals were kept under observation for one month and longer.

The results of all these inoculations were entirely negative both as to immediate and subsequent effects. At no time were there any symptoms or signs characteristic of an infection of the nervous system, the blood stream or the peritoneal cavity. The outcome was different when similar inoculations were practiced with cultures of streptococci or staphylococci obtained from various sources. The usual types of infection resulted and were generally fatal. In the rabbit, particularly, within twenty-four to thirty-six hours symptoms and signs of meningitis and abscess formation developed. At autopsy the gross findings were characteristic and the organisms were present in abundance at the site of inoculation and in the blood stream.

From these experiments it may be observed that the organism isolated and cultivated from poliomyelitic tissue, while capable of infecting and inducing experimental poliomyelitis in the monkey, is without effect in rabbits and smaller animals. These results, directly opposed to the findings of Rosenow and others, confirmed the belief that we were not dealing with an ordinary streptococcus or bacterium. In the monkey, moreover, inoculation of this culture was always identified with the typical incubation period of from six to eight days during which symptoms and signs were uniformly absent. Again, this result rules out a streptococcus or other organisms described by Rosenow and subsequent workers.

VIRUCIDAL POWER OF SERUMS FROM CONVALESCENT MONKEYS INOCULATED WITH CULTURES, AND RESISTANCE TO REINOCULATED POLIOMYELITIC VIRUS

Tests were made to compare the immunity response that is known to occur in typical experimental poliomyelitis with the results observed in monkeys that had been successfully inoculated with the culture. The question was concerned with whether or not an infection induced by the organisms would protect the animal against inoculation with the regular poliomyelitic virus. It has been shown that recovery from poliomyelitis induced by the filtered virus causes a state of resistance in monkeys. This is indicated by the power of the blood serum from convalescent monkeys to neutralize the virus filtrate.[10] Such animals also become insusceptible to reinoculation with virus.[46] Attempts were therefore made to determine whether monkeys in the convalescent stage following injection of cultures exhibited protection by either of these two reactions.

Neutralization in Vitro.—For this test the serums were taken from four monkeys. These had developed typical symptoms and paralyses following inoculation with cultures. Two monkeys had received cultures in the fourth subplant, and two other monkeys, the eleventh and the thirteenth subplant, respectively. Serum samples were withdrawn six to fourteen and one-half weeks after the initial symptoms and paralysis had appeared. The physical condition of two of these animals was fair; in two others there was advanced tuberculosis and marked cachexia.

Experiment 13.—The tests for neutralization were made by mixing 0.2 c.c. of a 5 per cent emulsion of poliomyelitis virus, glycerolated five days at 4° C., with 0.2 c.c. of each monkey serum, incubating for one hour at 37.5° C., and keeping for nine hours at 4° C. These mixtures which had been previously made up to a volume of one c.c. with sterile physiologic salt solution, were injected intracerebrally, under full ether anesthesia, into four normal Macacus rhesus monkeys. Two normal control animals received the virus suspension alone in the amounts of 0.1 c.c. and 0.05 c.c., respectively. This control material was subjected to the identical preliminary incubation and ice box fixation as were the serum-virus mixtures.

Macacus rhesus 78: February 24, 1932, intracerebral inoculation of mixture of 0.2 c.c. of 5 per cent virus suspension and 0.2 c.c. serum taken from Monkey 43, fourteen and one-half weeks after initial symptoms of poliomyelitis induced by a culture in the fourth subplant that had been passed through a second monkey. No symptoms; remained well.

Macacus rhesus 79: February 24, 1932, received mixture of virus suspension and serum taken from Monkey 58, on the forty-eighth day following first symptoms of poliomyelitis induced by inoculation of a culture in the eleventh subplant. No symptoms; remained well.

Macacus rhesus 80.—February 24, 1932, was injected into the cerebral hemisphere with a mixture of 0.2 c.c. of virus suspension and serum taken from Monkey 59, eight weeks after first symptoms of poliomyelitis following inoculation of a culture recovered from a monkey that had been injected with the original organisms in the fourth subplant. The animal remained free from symptoms until March 12, 17 days after the inoculation, when weakness of the right arm and both

legs were noted. The neck and back muscles were only slightly involved. Recovery of function progressed rapidly. Monkey 59 from which the serum was taken developed weakness of the left deltoid on the twelfth day following inoculation of the culture, and definite paralysis of the member four days later. There were no other effects and the recovery was rapid with complete restoration of function in the arm. The infection was very mild.

Macacus rhesus 81: February 24, 1932, intracerebral injection of 0.2 c.c. serum and 0.2 c.c. virus suspension. Serum was taken from Monkey 69 on the forty-first day following initial symptoms and paralysis induced by inoculation with a culture in the thirteenth subplant. This monkey was in very poor condition on this date and died on the following day. No symptoms; remained well.

Macacus rhesus 82: (Control) February 24, 1932, received 0.1 c.c. of a 5 per cent virus suspension into the cerebral hemisphere. March 1, bilateral ptosis, excitability, head tremor, and right facial paralysis were noted. March 2, the animal was prostrate and unable to move arms and legs.

Macacus rhesus 83: (Control) February 24, 1932, intracerebral inoculation of 0.05 c.c. of 5 per cent virus suspension. First symptoms were noted March 1; excitement, ruffled fur, and staccato cry. March 4, paralysis of arms and legs, ataxia and paralysis of muscles of trunk were observed. March 5, prostrate, and unable to move extremities.

Protection Tests.—Three monkeys were available of the four which had supplied the serums used in the neutralization experiment. These animals were now tested for protection against poliomyelitic virus introduced directly into the brain. A 5 per cent emulsion was prepared from the cord and brain of the same active virus that was used in the preceding tests. The material differed only with respect to an additional storage of three days in glycerol at 4° C. The dose of virus used was considerably larger, namely 0.3 c.c. to 0.4 c.c.

Monkey 43: Convalescent fourteen and one-half weeks. February 27, 1932, intracerebral inoculation of 0.3 c.c. of a 5 per cent virus suspension, glycerolated eight days at 4° C. No symptoms; remained unchanged.

Monkey 58: Convalescent forty-eight days. February 27, 1932, intracerebral injection of 0.4 c.c. virus suspension. No symptoms; remained unchanged.

Monkey 59: Convalescent eight weeks. February 27, 1932, intracerebral inoculation of 0.3 c.c. suspension of virus. No symptoms; no change.

Monkey 75: (Control) Normal animal. February 27, 1932, inoculated 0.3 c.c. of the same virus suspension into the left cerebral hemisphere. No symptoms developed until March 5, when the animal showed lessened activity and sat quietly in cage. March 7, weakness of arms, deltoid muscles and back were noted; inability to climb and fatigability were observed. There was a suggestive right facial paralysis and ptosis of right eyelid.

The results are summarized in Table III.

Comment.—These tests are significant in having demonstrated the neutralizing power for virus suspensions of serum taken from monkeys that have been inoculated with a culture of organisms capable of inducing the picture of typical poliomyelitic infection. These convalescent monkeys have likewise resisted an inoculation of poliomyelitic virus directly into the brain.

It may be deduced from this experiment that inoculation of the culture has caused in monkeys a resistance to poliomyelitic infection of the same nature and to a similar degree as that which is known to follow an infection with the ordinary virus.

Resistance to poliomyelitic infection has been demonstrated by means of this recognized test for serum neutralizing power and for protection against the effects of virus. It should be noted that the conditions imposed by this experiment have

TABLE III

NEUTRALIZATION TESTS WITH SERUM AND PROTECTION TESTS IN CULTURE-INFECTED MONKEYS

NO.	DATE	MATERIAL USED	AMOUNT C.C.	VIRUS AMOUNT SUSP. C.C.	RESULT
78	2/24/32	Serum, culture monkey, 14½ weeks convalescence	0.1	0.1	No symptoms
79	2/24/32	Serum, culture monkey, forty-eighth day convalescence	0.1	0.1	No symptoms
80	2/24/32	Serum, culture monkey, 8 weeks convalescence	0.1	0.1	Mild symptoms, seventeenth day
81	2/24/32	Serum, culture monkey, forty-first day convalescence	0.1	0.1	No symptoms
82	2/24/32	Control	–	0.1	Typical poliomyelitis, sixth day
83	2/24/32	Control	–	0.05	Typical poliomyelitis, seventh day
43	2/27/32	Culture monkey, 14½ weeks convalescence		0.3	No change
58	2/27/32	Culture monkey, forty-eighth day convalescence		0.4	No change
59	2/27/32	Culture monkey, 8 weeks convalescence		0.3	No change
75	2/27/32	Control		0.3	Poliomyelitis, seventh day

been purposefully made more stringent than are demanded by the established technic. A suspension of virus instead of the Berkefeld N filtrate has been used; the larger control monkeys have received half or less than half of the amount of virus inoculated into the test animals; the proportion of serum to the virus was 1:1 instead of the usual 10 to 100:1; finally the period of incubation and ice box fixation of the serum-virus mixtures was one hour and nine hours as against the usual periods of two hours and sixteen hours, respectively. Notwithstanding these rigorous requirements, the serum from three monkeys completely neutralized the larger doses of virus and that of the fourth animal delayed the appearance of a mild infection 12 days beyond the period in the controls. These were already paralyzed and prostrate on the sixth day after inoculation.

DISCUSSION

The results of the experiments described in the foregoing protocols indicate that poliomyelitis can be induced in monkeys by inoculation with certain organisms that have been cultivated from poliomyelitic nervous tissues. Successful inoculations in monkeys have resulted with visible cultures in the fourth to the eleventh, and also in the thirteenth subplants representing an extraordinary dilution of the original material cultivated. Koch's postulates have been fulfilled in all details including the recultivation of the identical organism with which poliomyelitis could again be reproduced in the experimental animal. Such recaptured "virus" possessed the original characteristics of filterability and pathogenicity.

This was shown by successful inoculations with Berkefeld filtrates prepared from the nervous tissues of monkeys that had been infected with a culture.

The theoretical possibility of original active virus being carried along with transfers of the culture material appears to be remote. Dilutions of the material as high as 2×10^{-27} would tend to exclude this and to confirm the belief that actual independent multiplication of an organism has occurred. The virulence of the culture could not be ascribed to the influence of virus as such upon remote subplants.

There remain for consideration also the theoretical possibility of adsorption of virus to the bodies of these organisms, and further, the multiplication of invisible virus in the tissue constituting the culture medium.

With regard to the first point, there is no explanation to account for the infectivity of the cultures except on the basic of their activity. If it were true that an invisible virus might be a constant companion of bacteria through an indefinite number of transfers, then there is no justification for the existence of bacterial etiology of disease. In yellow fever studies it has indeed been proved that a commonly associated organism cannot act as a vector for the virus.[47] As to the second point under consideration, the culture medium does not contain living tissue, and hence cannot be regarded as a possible nidus for the multiplication of a virus in its invisible state apart from the visible organisms that thrive in the sterilized tissue.

Of interest also in connection with the hypothetical survival of original virus in the cultures is the observed viability of the organisms for long periods of time at incubator temperature. From such cultures recent subplants retain infective power for monkeys.

The relation of streptococcal organisms to poliomyelitis has again been shown to be nonexistent. In the experiments reported here, only the disease recognized as experimental poliomyelitis in the monkey has been considered. Rabbits and smaller animals in contradistinction to the monkey, failed to develop any symptoms or signs of this disease when they were inoculated with the culture described in the foregoing protocols.

Although successful cultivation of an anerobic organism from poliomyelitis virus was accomplished by means of the EB medium in conjunction with the VB medium for subplants, further study will be necessary to establish the possible dependence of either one upon the other. For the recultivation of organisms from monkeys that had been successfully inoculated with the culture, the VB medium by itself generally was found satisfactory. However, the questions of adaptation of the strain, its multiplication, and maintaining of its virulence and infective power may be modified profoundly by the sequence of events taking place in the different culture mediums over varying periods of time. These matters must be left for future investigation as well as those recognized variable factors inherent in the culture mediums employed, undoubtedly a set of circumstances which affect the outcome of attempts to cultivate the virus of poliomyelitis.

SUMMARY AND CONCLUSIONS

Bacteriologic cultivation experiments were attempted with Berkefeld N filtrates prepared from the nervous tissues of monkeys inoculated with poliomyelitic virus and developing typical experimental poliomyelitis. Seven strains of virus

were studied in special culture mediums to be designated as EB and VB medium free from living tissue and containing essentially minced sheep brain in a veal infusion free from peptone.

Positive cultures were obtained from virus filtrates and the transfers from the original cultures have yielded successful growth in series. At the present time these organisms are in the twentieth subplant and the original cultures have been found viable after an incubation period of more than twenty-two weeks at 37.5° to 38° C.

The organism is a strict anerobe and grows in an atmosphere of reduced oxygen tension developed in the culture medium, stains best with Wright stain and in later subplants and older cultures it appears to be faintly to moderately gram-positive. Under a magnification of 1500 to 2000 diameters the organisms appear as minute ovoid bacteria ranging in size from the lowest limit of visibility to 0.05 –0.1 or 0.2 of a micron, and occur in irregular clusters, singly, in pairs, very rarely in short chains, and in densely packed masses. The bacterial bodies are especially numerous in the films of particulate brain tissue found in the medium, for which they exhibit a marked preference. A sheath-like envelop appears to surround the organism, favoring the formation of irregular masses.

Cultivation of the organism on the surface of a solid medium has also been successfully accomplished from subplants under strict anerobiosis by means of the Olitsky-Boëz apparatus. The culture medium devised for this purpose contained VB sheep brain in a base of buffered veal infusion agar, free from salt and peptone, and adjusted to P_H 7.6. The presence of abundant moisture was found essential for surface growth.

Inoculation in monkeys of organisms obtained from washed and unwashed cultures isolated from poliomyelitic tissues in VB medium caused clinical symptoms and signs and typical pathologic effects characteristic of experimental poliomyelitis. Berkefeld N filtrates as well as suspensions of nervous tissue from such infected animals were capable of inducing the identical infection in the normal monkey. The recaptured "virus" possessed the original characteristics of filterability, pathogenicity, and recultivation in an artificial medium.

Successful experiments up to the present time have been accomplished with these cultures in the third, fourth, eighth, ninth, eleventh, and thirteenth subplants, representing a dilution of approximately 2×10^{-7} to 2×10^{-27} of the original material cultivated. This, it will be noted, was 0.1 c.c. of a 5 per cent saline Berkefeld N filtrate prepared from poliomyelitic nervous tissue. Parallel experiments in which heated cultures were used for inoculation into monkeys resulted negatively. Materials derived from culture tubes that contained no demonstrable organisms were also without effect. Additional control experiments included material from normal monkeys, and from the tissues of herpes, encephalitis and neurovaccinia. All these, as well as the uninoculated culture medium by itself gave negative results.

Attempts to recultivate the typical organism from monkeys experimentally infected with the culture and developing poliomyelitis, were successful with Berkefeld N filtrates and suspensions prepared from the nervous tissues of such animals. With such recaptured virus cultures the typical picture of experimental poliomyelitis was again induced in normal monkeys. Successful transmission of the

disease from these animals to normal ones was again accomplished by means of Berkefeld N filtrates and suspensions of nervous tissues.

The inoculation of rabbits, guinea pigs, and mice with the culture has resulted negatively. Direct injection of massive doses into the brain, or into the blood stream, or peritoneal cavity was uniformly without effect.

Film preparations with material taken at autopsy from the site of inoculation and from the surface and deeper layers of the cord and brain of monkeys were uniformly negative for organisms. Inoculation of such materials into ordinary culture medium gave negative results. The same observations were made in rabbits, guinea pigs and mice.

Serums taken from monkeys that have been inoculated with the culture of organisms capable of inducing the picture of typical poliomyelitic infection were found to possess neutralizing power for active virus suspensions. Such convalescent monkeys likewise resisted an inoculation of poliomyelitic virus into the brain. The resistance to poliomyelitic infection induced by the inoculation of the culture was the same as that which is known to follow an infection with the filtered virus or its suspension.

Filtration experiments with the cultures during certain stages of growth suggest that ultramicroscopic forms may be capable of developing into visible bodies in the culture medium.

The cycle of poliomyelitis has been demonstrated in monkeys inoculated with cultures. The organism has been developed from an invisible to a visible state and the typical disease induced in the experimental animal, in the brain and spinal cord of which the organism again returns to the invisible filterable stage, but retains its power to infect normal monkeys.

It appears from these studies that the poliomyelitic virus has multiplied in vitro in a culture medium containing nonliving brain tissue. The organism causes pathologic effects that can be augmented by successive passage of the culture material through a series of monkeys. Notable in this respect is the shortening of the incubation period.

The organism exhibits a marked resistance to the action of 50 per cent glycerol over long periods of time.

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RESPIRATORY METABOLISM AND PULMONARY VENTILATION IN PULMONARY TUBERCULOSIS*†

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THERE appear in the literature from time to time reports of increased basal metabolism in tuberculosis although the majority of workers have found the metabolism to be approximately normal except in the presence of fever. Can these conflicting reports be due to differences in the amount or character of the disease other than fever, or are the high values only reports of basal metabolism which was not really basal?

It is very easy to obtain high rates and to be misled into believing the results to be basal because the patient was quiet and the pulse and respiration stationary. Only repeated tests will prove that the first ones were too high and that the patients were not sufficiently relaxed to give the minimal rate.

Pulmonary ventilation has been observed to be higher in tuberculous subjects than in normal subjects; and one cause to which it has been attributed is lowered vital capacity. This work was undertaken in an attempt to determine whether the basal metabolism in a series of tuberculous patients could be related either to the extent or to the intensity of the disease and to determine whether this observed increase in pulmonary ventilation is the result simply of the amount of the destruction of lung tissue, or whether it is in some way more closely related to the toxic condition of the subject. In other words, is it related to the extent or to the progression of the disease?

The study also included observations on the relative increase in the ventilation as the oxygen consumption was raised. This increase could not be too large due to the limited size of the spirometer available; hence the respiratory exchange after food was chosen for study.

It was the plan when this problem was begun to study the pulmonary ventilation and oxygen consumption (from which the basal metabolism could be calculated) in both toxic and nontoxic cases, and in the latter to relate each to the extent of involvement. Unfortunately, due to lack of time, only studies on nontoxic cases were carried out. Consequently, this is a report of studies made of the pulmonary ventilation and oxygen absorption in the basal condition and again after breakfast, of nontoxic cases of pulmonary tuberculosis and similar studies upon a series of normal subjects.

TECHNIC

A Benedict-Roth metabolism machine and a Haldane gas analyzer were used for the determinations. The Roth was used both as a metabolism machine and also, by making a few simple adjustments, as a simple spirometer for the collection of expired air.

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The usual procedure for basal metabolism was observed. The ambulatory patients, without food since the previous evening, came to the metabolism room and rested on a cot for three-quarters of an hour, and the infirmary patients, also without food for twelve to fourteen hours, rested quietly in their rooms for half an hour. The test was not begun until pulse and respiration had reached a constant level, and during the test the rate and character of the respiration were noted, as it is essential for accurate observations that they do not change when expiration into the spirometer is taking place.

During the first part of the test, a determination was made of the oxygen consumption, as in a regular basal metabolism test, for a period of seven to eight minutes. The second part consisted of a collection of expired air. As the spirometer was too small for a continuous collection of several minutes, after approximately one minute (time noted exactly in seconds in each instance) a three-way valve was turned, at the end of an expiration, so that the subject was breathing out into the room. Readings of the volume of expired air in the spirometer and the time of collection were recorded, a sample of the expired air was collected in a sampling tube as part of the composite sample for later analysis, and the spirometer was emptied. This procedure required about thirty seconds. Then the valve was turned again at the end of an expiration allowing the subject to expire into the spirometer and a second collection was made. This procedure was repeated eight or ten times. The patient then had breakfast, returned for a rest of three-quarters of an hour and repeated the experiment which began usually one hour after the end of the meal.

To guard against experimental error, tests were repeated until the subject gave consistent results. Frequently, as is the case in all basal metabolism and respiration work, the first test or two had to be discarded because the results were obviously not correct. Sometimes in the first test the subjects were not completely relaxed, due possibly to slight apprehension about the whole procedure, and their metabolism as a result was too high. Overventilation also, was very likely to occur the first time a subject breathed into the spirometer. Work on two subjects had to be discontinued entirely because they never became sufficiently accustomed to the machine to breathe normally when connected with it.

The composite sample of expired air, which contained equal parts of each spirometer of expired air, was analyzed for both CO_2 and O_2. Duplicate analyses were always made and the CO_2 required to check within 0.04 per cent (and usually did within 0.02) and the O_2 to check within 0.05 per cent. Analyses of outside air were made regularly, almost daily, to check the machine.

If there was a difference of more than 5 c.c. per minute between the oxygen as determined in the first part of the test and as determined by the analysis of expired air, the volume of the latter was corrected to make the two agree since the first was considered the more reliable test, because of the intermittent collection of expired air. When occasionally such a correction was found necessary, it was small, since special care had been taken to see that the nature of the respiration during the observation was normal.

The basal metabolism was calculated on the basis of calories per square meter of surface area and compared with the Aub-DuBois (1917) standards. The calculation of the oxygen absorption and carbon dioxide elimination from the analysis

of expired air was made as described by Bailey (1921). Correction factors were taken from the tables compiled by Carpenter (1924).

SUBJECTS

The group of subjects studied consisted of eight normals and nineteen tuberculous patients in the sanatorium, eleven of the latter with moderately advanced and eight with far advanced lesions. Of the normals, three were medical students, two secretaries, one a laboratory worker, one a teacher, and one a nontuberculous pleurisy patient.

The moderately advanced patients (with one exception, Case 19) were ambulant at the time they were studied although subsequently four of them, Cases 9, 11, 13, and 17, had increase in infiltration or reactivation of the disease and were put to bed. The duration of the disease had been less than a year in all except Case 14 who had had a recent fresh exacerbation of an old lesion of several years' standing.

Of the eight far advanced cases three were bed patients and their disease was of recent origin. Of the five ambulatory cases, Cases 20 and 21 had each had tuberculosis for about a year and a half and Case 27 had "cured" several years previously.

All the subjects in both groups were afebrile and generally nontoxic at the time they were studied.

The average age was only slightly different for the three groups. In the normals all subjects were between twenty and thirty-five years of age with an average age of twenty-six and a half. The moderately advanced cases were between twenty-one and forty-six years of age with an average of twenty-nine and the far advanced cases between eighteen and thirty-six with an average of twenty-nine years.

The tuberculous subjects were classified according to the National Tuberculosis Association classification and in addition on the x-ray findings alone. According to the extent of the parenchymatous (Pc) lesion they were divided into stages

			MODERATELY ADVANCED			
Case 9	Pc2	L	4r and 6 vs c_2			
Case 10	Pc2	R	3r and 5 vs; 5r and 6 vs; 7r and 8 vs b_1			
		L	3r and 5 vs a_0			
Case 11	Pc2	R	3r and 5 vs b_2	L	6r and 9 vs c_2	
Case 12	Pc2	R	4r and 7 vs $c?_2$			
Case 13	Pc2	R	3r and 5 vs a_1	L	2r and 4vs; 4 and 6r; 7 and 9 vs c_1	
Case 14	Pc2	R	2r and 4 vs b_1	L	3r and 6 vs c_1	
Case 15	Pc2	R	2r and 4 vs b_2	L	3r and 5 vs b_2	
Case 16	Pc2	R	1r and 3 vs and lower ½ b_1			
Case 17	Pc2	R	4r and 5 vs b_1	L	4r and 5 vs $c?_1$	
Case 18	Pc2	R	4r and 6 vs c_2			
Case 19	Pc2	R	3r and 5 vs c_2	L	clav. and 3 vs a_1	
			FAR ADVANCED			
Case 20	Pc3	R	5r and 7 vs c_2	L	5r and 7 vs c_2	
Case 21	Pc3	R	6r and 9 vs c_1	L	3r and 5 vs b_1	
Case 22	Pc3	R	6r and 9 vs b_2	L	4r and 6 vs b_2	
Case 23	Pc3	R	4r and 6 vs c_1	L	4r and 9 vs b_1	
Case 24	Pc3	R	5r and 9 vs c_1	L	5r and 9 vs b_1	
Case 25	Pc3	R	6r and 9 vs c_2	L	5r and 7 vs b_2	
Case 26	Pc3	R	5r and 9 vs c_2	L	6r and 9 vs c_2	
Case 27	Pc3	R	4r and 7 vs b_1	L	4r and 7 vs b_1	

2 and 3, which correspond to the National Tuberculosis Association classification of "moderately advanced" and "far advanced." The side and extent on each side, measuring from the apex down to the specified rib or vertebral spine, are recorded with the intensity of the lesion, a, b, or c. The probability of focal change or activity is expressed in suffixes 0, 1, or 2 for each side.

BASAL METABOLISM

The results of the experiments are grouped in two sets of tables, the first dealing with the respiratory metabolism and the second with the pulmonary ventilation. Tables I, II, and III list the metabolism in calories per square meter of body surface per hour together with the percentage deviation from the normal value according to the Aub-DuBois standards. The values after breakfast, with the percentage increase and also the respiratory quotients are given.

In order to shorten the tables, individual experiments have not been listed. As has been stated previously, tests were made until consistent results were obtained and the first experiments were discarded if the results were higher than that found in subsequent tests. Any other experiments were discarded also if for any reason they were not basal. The results given in the tables are the average result of the consistent tests and the column entitled number of tests, represents the number averaged and not necessarily the number actually made upon the subject.

A survey of the tables reveals the fact that of the 27 cases, 25 had a basal metabolism below the expected value. In the normal group there were no values which were higher than the standard and two subjects had values of –16.5 per cent and –19 per cent. The entire group had an average of –9 per cent which, however, falls within the limits of normal variation even if we retain the narrower limits of ±10 per cent.

To make certain that the machine was not giving too low oxygen values, a sec-

TABLE I

RESPIRATORY METABOLISM OF NORMAL SUBJECTS

SUBJ. SEX	NO. TESTS	NUTRITIVE CONDITION	EXPECTED CAL./SQ.M./HR. AUB-DUBOIS	FOUND CAL./SQ.M. PER HR.	B.M.R.	INCR. AFT. BREAKFAST PER CENT	R.Q.
1 F	2	Basal	36.5	33.6	– 7.9		0.89
	1	1 hr. aft. break.		37.5		+11.6	0.91
2 F	2	Basal	37.0	30.9	–16.5		0.73
	2	1 hr. aft.break.		33.1		+ 7.1	0.84
3 F	2	Basal	37.0	34.4	– 7.0		0.71
	2	1 hr. aft. break.		39.3		+14.2	0.87
4 M	2	Basal	39.5	39.1	– 1.0		0.80
	2	1¼ hr. aft. break.		43.1		+10.2	0.82
5 M	2	Basal	39.5	35.7	– 9.4		0.86
	2	1 hr. aft. break.		41.6		+16.5	0.85
6 M	2	Basal	39.5	32.0	–19.0		0.90
	2	1 hr. aft. break.		37.3		+16.5	0.88
7 F	2	Basal	37.0	33.9	– 8.4		0.79
	2	1 hr. aft. break.		38.1		+12.4	0.80
8 F	2	Basal	37.0	35.9	– 2.8		0.76
	2	1 hr. aft. break.		39.8		+10.8	0.88
Average		Basal	37.6	34.4	– 9.0		0.81
		1 hr. aft. break.		38.7		+12.4	0.86

ond portable apparatus of a different make was procured and with a subject thoroughly accustomed to the metabolism tests, alternate determinations were made, first on one machine and then on the other. Four tests, two on each machine, made within the course of an hour, checked within 1.5 absolute per cent. Hence, there is every reason to believe that the low metabolism values are absolutely correct.

TABLE II

RESPIRATORY METABOLISM OF MODERATELY ADVANCED TUBERCULOUS SUBJECTS

SUBJ. SEX	NO. TESTS	NUTRITIVE CONDITION	EXPECTED CAL./SQ.M./HR. AUB-DUBOIS	FOUND CAL./SQ.M. PER HR.	B.M.R.	INCR. AFT. BREAKFAST PER CENT	R.Q.
9 F	2	Basal	37.0	30.0	−18.9		0.84
	2	1¾ hr. aft. break.		35.1		+17.0	0.86
10 M	2	Basal	39.5	36.0	− 8.8		0.78
	2	1 hr. aft. break.		39.2		+ 8.9	0.89
	1	1½ hr. aft. break.		38.9		+ 8.0	0.90
11 F	1	Basal	37.0	36.1	− 2.4		0.83
	1	1 hr. aft. break.		38.1		+ 5.8	0.92
12 M	1	Basal	39.5	40.7	+ 3.0		0.83
	3	1 hr. aft. break.		44.7		+ 9.8	0.87
	1	1½ hr. aft. break.		42.2		+ 3.6	0.85
13 F	2	Basal	37.0	34.0	− 8.2		0.82
	2	1 hr. aft. break.		45.2		+32.9	0.86
	2	2 hr. aft. break.		40.4		+18.8	0.93
14 M	1	Basal	38.5	37.0	− 3.9		0.87
	2	1 hr. aft. break.		43.9		+18.6	0.86
15 M	2	Basal	39.5	34.1	−13.9		0.83
	2	1 hr. aft. break.		37.9		+11.1	0.92
	1	2½ hr. aft. break.		36.1		+ 5.8	0.88
16 F	2	Basal	36.0	36.5	+ 1.4		0.82
	2	1 hr. aft. break.		40.7		+11.5	0.94
	1	1½ hr. aft. break.		38.0		+ 4.1	0.81
17 M	2	Basal	39.5	33.5	−15.3		0.82
	3	1 hr. aft. break.		39.0		+16.4	0.85
18 M	2	Basal	39.5	34.7	−12.1		0.83
	2	1 hr. aft. break.		42.2		+22.8	0.82
19 M	2	Basal	39.5	31.9	−19.2		0.83
	2	1 hr. aft. break.		39.2		+22.9	0.88
Average		Basal	38.4	34.9	− 8.9		0.83
		Basal minus No. 9	38.6	35.4	− 7.9		0.83
		1 hr. aft. break.		41.0		+16.2	0.88

In the group of moderately advanced cases there were only two positive values, one of +3 per cent and one of +1.4 per cent while there were five with values below −10 per cent. The average for the group was −7.9 per cent.

Even in the far advanced group there were no high rates. Three of the eight cases had values below −10 per cent and the average was −8.0 per cent.

Of the nineteen tuberculous patients there were three whose basal metabolism was between −15 per cent and −20 per cent; five between −10 per cent and −15 per cent and nine between 0 per cent and −10 per cent making seventeen in all that were below the expected value.

Lack of normal muscular activity over a considerable time fails to explain the low values because several cases with low metabolism had been as active as usual only a few weeks prior to the tests.

TABLE III

RESPIRATORY METABOLISM OF FAR-ADVANCED TUBERCULOUS SUBJECTS

SUBJ. SEX	NO. TESTS	NUTRITIVE CONDITION	EXPECTED CAL./SQ.M./HR. AUB-DUBOIS	FOUND CAL./SQ.M. PER HR.	B.M.R.	INCR. AFT. BREAKFAST PER CENT	R.Q.
20 M	2	Basal	39.5	34.3	−13.1		0.78
	2	1 hr. aft. breakfast		37.1		+ 8.2	0.86
21 M	3	Basal	39.5	37.2	− 5.8		0.84
	4	1 hr. aft. breakfast		47.7		+28.2	0.89
	1	1½ hr. aft. breakfast		42.4		+13.9	0.87
22 F	2	Basal	36.5	35.5	− 2.7		0.82
	2	1 hr. aft. breakfast		45.3		+27.6	0.95
	1	1½ hr. aft. breakfast		43.8		+23.1	0.88
23 F	2	Basal	36.5	31.8	−12.9		0.83
	2	1 hr. aft. breakfast		35.9		+12.9	0.88
24 M	2	Basal	39.5	36.6	− 7.3		0.87
	2	1 hr. aft. breakfast		42.5		+16.1	0.94
25 M	2	Basal	39.5	38.5	− 2.5		0.78
	2	1 hr. aft. breakfast		45.8		+19.0	0.85
26 F	3	Basal	38.0	36.1	− 5.0		0.83
	3	1 hr. aft. breakfast		39.2		+ 8.6	0.87
27 M	2	Basal	39.5	33.7	−14.7		0.86
	2	1 hr. aft. breakfast		38.6		+14.5	0.88
Average		Basal	38.6	35.5	− 8.0		0.83
		1 hr. aft. breakfast		41.5		+16.9	0.89

Thus in these studies of afebrile nontoxic cases there was not the slightest evidence of an increased basal metabolism in either group. The rate in the series of far advanced cases was equally as low as for the moderately advanced series and as low as in a similar group of normal subjects which makes it appear that basal metabolism in the tuberculous person bears no relationship to the *amount* of the involvement; i. e., to extent of lesion.

Brock and Haskins (1927) also obtained low basal metabolism values for several cases which correspond with the values reported for this work, a −14 per cent for the average of three patients studied over several months during the summer when our cases were studied.

The majority of workers find the basal metabolism to be approximately normal for minimal tuberculous subjects. Among the more recent workers holding that view are Lanz (1925), Giegler (1927), Frank and Safarik (1928), Salus and Adler (1928), and McMahon and Klein (1929). McBrayer (1921), on the other hand, finds in 61 per cent of his "incipient" tuberculous subjects (22 cases) a basal metabolic rate greater than normal, a percentage which is even slightly higher than for a group of 21 advanced cases in which 57 per cent were above normal.

Concerning the metabolism in advanced cases there is more disagreement. For example, Gekler and Weigel (1926) from their study of eighty cases conclude that the toxemia of tuberculosis does not increase the basal metabolic rate and that increased rates are to be associated with hyperthyroidism, while Giegler (1927) with one hundred thirty cases reports normal values only for minimal tuberculosis with higher values for advanced cases, the rate corresponding to the grade of activity.

Cordier (1923) reports normal basal metabolism in afebrile cases; but Hyge (1928) finds increased rates in the third stage even when afebrile, although he

states that when the patient is in good condition clinically the metabolism is usually normal.

McCann and Barr (1920) in their careful study of tuberculous patients in the calorimeter of the Russell Sage Institute found basal values for ten cases to be between −3 per cent and +15 per cent with an average value of +8 per cent. With fever, however, the metabolism was increased; and, with a rectal temperature of 40° C., the authors find it may go as high as 30 per cent above normal.

Williamson (1929) reports average values of +23 per cent and +18 per cent for two groups with pyrexia or other toxic symptoms and −0.5 per cent and −1.0 per cent for apyretic groups. McMahon and Klein (1929) with a group of forty active cases who had fever during the day but were afebrile in the morning when the tests were made give the following average figures for basal metabolism; incipient cases, +3.9 per cent; moderately advanced cases, +13 per cent; and far advanced cases, +21 per cent.

The greatest increases are reported by Gräfe (1920), who in seven of ten afebrile acute tuberculous subjects found increases of from 20 to 36 per cent, and in three febrile (over 39° C.) cases, increases from 50 to 75 per cent. His values undoubtedly are not basal. The determinations were made in a Jacquet chamber over periods several hours in length and such results cannot be compared with short periods during which the subject can remain absolutely quiet and relaxed.

INFLUENCE OF FOOD

The chief interest in the study of the metabolism after breakfast was not in the specific dynamic action of the food, but rather in the relationship between the increase in the oxygen consumption and the increase in the pulmonary ventilation. This relationship will be discussed later in the section on pulmonary ventilation.

In these experiments, since it was not feasible to have each subject given the same breakfast and in equal amounts, the results upon each individual cannot be compared one with the other. The average values for the groups are, however, not without interest.

In the normal group the metabolism, one hour after the meal, showed increases varying from 7.1 per cent to 16.5 per cent with an average of 12.4 per cent. The average respiratory quotient rose from 0.81 to only 0.86 due, undoubtedly, to the relatively large amounts of protein and fat in the meal.

The moderately advanced group showed wide variations from 5.8 per cent in one case to 32.9 per cent in another. The average increase was 16.2 per cent and was practically the same for the men and the women. The respiratory quotient rose from 0.83 to 0.88.

The individual variation within the group of far advanced cases was nearly as great, 8.2 per cent to 28.2 per cent; and the average increase is the same as for the moderately advanced group, i. e., 16.9 per cent. The respiratory quotient also shows the same rise, 0.83 to 0.89.

Since the amount of food eaten in each case was not controlled, it is impossible to tell whether or not this slightly greater increase which was observed in the tuberculous groups is a real difference due to the disease. It is not great enough to be very significant at any rate; and too, both McCann (1921) and Salus and Adler

(1928) report a normal response to food in the tuberculous. The latter authors found no relationship between specific dynamic action and activity in the lungs and the writer's results would tend to bear out this conclusion.

PULMONARY VENTILATION

In comparing the pulmonary ventilation, oxygen consumption and carbon dioxide production of one group of subjects with another, there arises the difficulty of individual variations due to size, sex, and age. It is definitely known that the oxygen and carbon dioxide are affected by all three factors and it is most probable that the ventilation is likewise influenced. This is borne out by the findings of several workers (Krogh and Lindhard, 1913; Boothby, 1915; Pearce, 1921) that pulmonary ventilation varies directly with the oxygen consumption.

In computing basal metabolism, this difficulty is eliminated by expressing the calories on the basis of surface area of the body and comparing them with standards which take account of differences due to sex and age. But for pulmonary ventilation we have no such standards. In this work the age factor may be eliminated since the average age of each group was nearly the same. The results may then be expressed on the basis of surface area or they may be arranged according to sex, with a weighted average depending upon the number of males and females in the group.

The ventilation values in this work have been calculated by both these methods and, curiously enough, the variations for the three groups of subjects were of the same order of magnitude whichever way they were expressed. This is probably

TABLE IV

PULMONARY VENTILATION OF NORMAL SUBJECTS

SUBJ. SEX	NO. TESTS	NUTRITIVE CONDITION	EXPIRED AIR LITERS/MIN.	INCR. %	CO_2/O_2 C.C. MIN.	INCR. IN O_2 %	PULSE	RESP.
1 F	2	Basal	5.84		160/179		50	13
	1	1 hr. aft. break.	6.27	7.4	182/199	10.0	56	16
2 F	2	Basal	4.08		120/164		68	16
	2	1 hr. aft.break.	3.97	− 2.8	147/177	7.9	70	16
3 F	2	Basal	4.86		125/176		74	12
	2	1 hr. aft. break.	5.57	14.6	175/201	14.2	82	14
7 F	2	Basal	5.88		161/203		75	14
	2	1 hr. aft. break.	6.13	4.3	180/226	11.3	79	16
8 F	2	Basal	5.55		155/203		81	14
	2	1 hr. aft. break.	6.60	18.9	198/227	11.4	88	15
Average for F		Basal	5.24		144/185		70	14
		1 hr. aft. break.	5.70	8.5	176/205	10.9	75	15
4 M	2	Basal	5.61		209/262		52	8
	2	1¼ hr. aft. break.	6.02	7.3	238/290	10.7	52	10
5 M	2	Basal	6.81		220/255		52	11
	2	1 hr. aft. break.	7.51	10.3	252/297	16.4	64	13
6 M	2	Basal	5.99		180/199		50	11
	2	1 hr. aft. break.	6.30	5.2	204/230	15.5	58	12
Average for M		Basal	6.14		203/238		52	10
		1 hr. aft. break.	6.61	7.6	231/272	14.2	58	12
Mean		Basal	5.69		173/211		61	12
		1 hr. aft. break.	6.16	8.1	203/238	12.6	66	13

TABLE V

PULMONARY VENTILATION OF MODERATELY ADVANCED TUBERCULOUS SUBJECTS

SUBJ. SEX	NO. TESTS	NUTRITIVE CONDITION	EXPIRED AIR LITERS/MIN.	INCR. %	CO_2/O_2 C.C. MIN.	INCR. IN O_2 %	PULSE	RESP.
11 F	1	Basal	5.31		168/202		84	11
	1	1 hr. aft. break.	6.28	18.3	197/214	5.9	84	12
13 F	2	Basal	5.24		168/204		76	18
	2	1 hr. aft. break.	6.92	32.1	234/269	31.9	88	20
	2	2 hr. aft. break.	6.70	27.9	222/239	17.1	76	20
16 F	2	Basal	5.18		157/190		72	16
	2	1 hr. aft. break.	6.02	16.2	205/212	11.5	72	16
	1	2 hr. aft. break.	5.16	0.0	160/197	3.7	80	16
Average for F		Basal	5.24		164/199		77	15
		1 hr. aft. break.	6.41	22.2	212/231	16.4	81	16
10 M	2	Basal	5.02		175/217		70	11
	2	1 hr. aft. break.	5.49	9.4	209/236	8.8	80	12
	1	1½ hr. aft. break.	5.38	7.1	211/234	6.0	80	11
12 M	1	Basal	7.46		235/282		68	18
	3	1 hr. aft. break.	8.38	12.3	272/310	9.9	76	19
	1	1½ hr. aft. break.	8.08	8.3	249/293	3.7	72	16
14 M	1	Basal	6.52		211/243		68	9
	2	1 hr. aft. break.	7.00	7.4	247/284	16.9	72	14
15 M	2	Basal	5.60		173/209		64	12
	2	1 hr. aft. break.	6.78	21.1	216/232	11.0	64	12
	1	2½ hr. aft. break.	6.20	10.7	192/220	5.3	64	12
17 M	2	Basal	5.72		176/216		80	15
	3	1 hr. aft. break.	6.61	15.6	215/253	17.1	80	17
18 M	2	Basal	5.56		186/226		78	6
	2	1 hr. aft. break.	6.06	9.0	225/275	21.7	84	4
19 M	2	Basal	5.37		177/215		74	13
	2	1 hr. aft. break.	6.23	16.0	232/264	22.7	86	11
Average for M		Basal	5.89		190/230		72	12
		1 hr. aft. break.	6.65	13.0	231/264	15.4	77	13
Mean		Basal	5.57		177/215		75	13
		1 hr. aft. break.	6.53	17.6	222/249	15.9	79	14

pure coincidence for this series. Since it was simpler to arrange the values according to sex, that method was adopted and Tables IV, V, and VI give separate averages for the men and for the women, of the pulmonary ventilation, of the percentage of CO_2 in the expired air, of the CO_2 and O_2 in c.c. per minute, as well as of the pulse and respiration rates. At the foot of the tables is given the mean of the two averages.

From the tables it will be seen that, in the basal condition, the pulmonary ventilation varies for women between 4.08 and 5.84 liters per minute, and for men between 5.99 and 6.81 liters, the weighted average being 5.69. In the group of moderately advanced cases the values are nearly identical for the three women, i. e., 5.24 liters, and vary for the men between 5.02 and 7.46. The average for the entire group is 5.57 liters which is even slightly less than that of the normals. In the far advanced group the variation among the women is between 5.42 and 6.22 and among the men between 5.11 and 7.23, the group average being 6.05 liters per minute.

Evidently then, the ventilation, in the basal state, as well as the oxygen consumption, can remain practically normal even with considerable involvement of

TABLE VI

PULMONARY VENTILATION OF FAR ADVANCED TUBERCULOUS SUBJECTS

SUBJ. SEX	NO. TESTS	NUTRITIVE CONDITION	EXPIRED AIR LITERS/MIN.	INCR. %	CO_2/O_2 C.C. MIN.	INCR. IN O_2 %	PULSE	RESP.
22 F	2	Basal	5.42		160/195		84	12
	2	1 hr. aft. break.	7.08	30.6	232/248	27.2	88	13
	1	1½ hr. aft. break.	6.75	24.5	213/242	24.1	92	11
23 F	2	Basal	6.22		167/202		82	13
	2	1 hr. aft. break.	7.34	18.0	201/229	13.4	86	13
26 F	3	Basal	5.69		158/188		84	13
	3	1 hr. aft. break.	5.83	2.5	175/201	6.8	99	12
Average F		Basal	5.78		162/195		83	13
		1 hr. aft. break.	6.75	17.0	203/226	15.8	91	13
20 M	2	Basal	5.11		158/205		78	13
	2	1 hr. aft. break.	6.02	17.8	188/219	6.8	82	13
21 M	3	Basal	7.23		221/264		83	9
	4	1 hr. aft. break.	9.00	24.5	304/338	28.0	86	11
	1	1½ hr. aft. break.	7.63	5.5	262/300	13.6	80	9
24 M	2	Basal	6.90		207/238		70	13
	2	1 hr. aft. break.	8.02	16.2	258/276	16.0	92	13
25 M	2	Basal	5.43		179/231		116	9
	2	1 hr. aft. break.	6.74	24.1	232/275	19.0	120	11
27 M	2	Basal	6.97		187/217		62	13
	2	1 hr. aft. break.	7.90	13.3	221/249	14.8	84	15
Average M		Basal	6.33		190/231		82	11
		1 hr. aft. break.	7.54	19.2	240/271	16.3	93	13
Mean		Basal	6.05		176/213		83	12
		1 hr. aft. break.	7.15	18.1	222/249	17.1	92	13

lung tissue. The value in the far advanced series is only 6 per cent above that of the normals and is, undoubtedly, within the limits of normal variation. Certainly it is very insignificant in view of the increase which McCann (1921) found of nearly 100 per cent for five toxic cases. His subjects were all in an advanced stage of the disease, much emaciated and with considerable fever during the day although not particularly high in the morning when the observations were made, the average rise above normal being 1.3° F. His average value for five normals was 4.255 liters per minute, which is less than was found in this series of normals; but the average for his tuberculous cases was 8.249 liters, which is 94 per cent above his normal value and 45 per cent above the normal value of this series.

There is then this striking difference in the amount of the pulmonary ventilation between toxic and nontoxic cases. The amount of the involvement was undoubtedly greater in the cases which McCann studied but surely not enough greater to account for the large difference found, since the nontoxic cases, even the ones with lesions sufficiently extensive to place them as far advanced cases, had a normal ventilation.

It is impossible to say whether this difference is due to a greater reduction of vital capacity as McCann suggested or not, since, unfortunately, there are no vital capacity determinations in either series of studies. It is quite possible that this is so because there is considerable evidence indicating that vital capacity becomes progressively lower as the disease advances (Myers, 1925). On the other hand, the lowered vital capacity may be only one of several factors present in the toxic state

which are affecting the pulmonary ventilation and only further work can give us the answer to that.

If we turn now to a consideration of the increase in ventilation following the breakfast, we find considerable variation in all three series, as we should expect with the diet uncontrolled. In the normals, however, the increases never reached the heights that they did in some of the tuberculous subjects. With one normal, Case 2, the respiratory exchange after the meal was slightly less than before, although the oxygen consumption was raised 8 per cent.

As was brought out in the section dealing with the metabolism after breakfast, individual figures cannot be compared because of lack of uniformity of the breakfasts; but the average value for the three series is of some interest, since the average amount of food eaten in each group would probably be approximately the same. We find that the normals increased their ventilation only 8 per cent, while the tuberculous groups increased theirs 18 per cent.

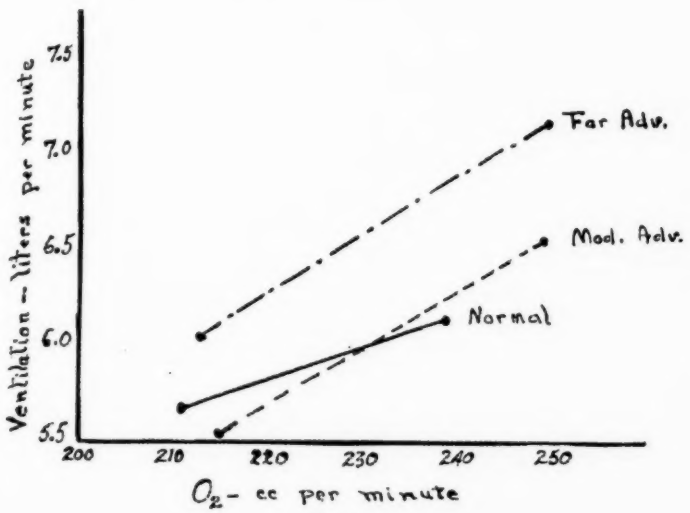

Fig. 1.

The significant point in this connection is the relative increase in ventilation and in oxygen consumption. The normals, in order to increase their oxygen intake 12.6 per cent, needed to increase their ventilation only 8.1 per cent, whereas the moderately advanced groups in order to increase their oxygen 15.9 per cent needed to increase their ventilation 17.6 per cent; and the far advanced group increased their oxygen 17.1 per cent and their ventilation 18.1 per cent. This is shown graphically in Fig. 1, in which the basal and after food values for oxygen are plotted against the corresponding values for ventilation. The slope of the curve for the tuberculous groups is definitely steeper than that for the normal group.

All three groups had a slight rise in pulse rate after breakfast; but the respiratory rate was, on the average, only one point higher than before breakfast.

Thus it appears that a patient may have extensive involvement in both lungs, but, if the disease is of sufficiently low virulence or the resistance of the body is sufficiently high so that no toxic symptoms make their appearance, the lungs are still

able, with their normal amount of ventilation, to supply the body with the necessary amount of oxygen in the basal condition, that is, the minimal requirement of oxygen. This mechanism, however, must be working at nearly its maximum since a small increase in oxygen consumption of only 17 per cent caused in the tuberculous subjects an equal or slightly greater percentage increase in ventilation, whereas the normal subjects increased their ventilation by less than two-thirds the amount of their percentage increase in oxygen.

This corroborates the findings of Williamson (1928) who reported for a series of observations on one normal and on four tuberculous patients the O_2 consumption and pulmonary ventilation values for basal conditions, again at 11:15 A.M., and at 5:15 P.M., after tea. His values for basal O_2 for the eight determinations on the normal varied between 197 and 252 c.c. per minute and the pulmonary ventilation between 4.76 and 6.09 liters per minute. Since we know that the basal metabolism usually varies within rather narrow limits (Lusk 1928), these variations of as much as 28 per cent are rather disturbing; but if we calculate the percentage increases of O_2 and ventilation which he found at 11:15 A.M. and again after tea at 5:15 P.M. we find exactly the same relation that the writer finds, namely: that, in the normal the percentage increase in pulmonary ventilation is only about two-thirds of the percentage increase in O_2 consumption. His increases were 21 and 35 per cent for the O_2 and 15 and 22 per cent respectively for the ventilation. The tuberculous patients, on the other hand, needed to increase their ventilation slightly in excess of the increase in O_2. Increases of 15 and 22 per cent in the O_2 called forth increases in the ventilation of 17 and 27 per cent respectively.

This is only a small difference; but if we realize that this difference is the result of a rise in O_2 consumption of approximately 20 per cent and that a man walking down the street at a moderate pace can double or more than double his O_2 absorption (Benedict and Murchhauser, 1915), this difference becomes a significant factor in the dyspnea of many tuberculous subjects following the slightest exertion.

SUMMARY

1. With a Benedict-Roth metabolism machine and a Haldane gas analyzer the respiratory metabolism and pulmonary ventilation were determined before breakfast (basal) and again one hour after breakfast on eight normal and nineteen tuberculous subjects. The latter were composed of eleven with moderately advanced pulmonary lesions and of eight with far advanced pulmonary lesions; but all were afebrile, nontoxic patients and fifteen were ambulant.

2. The basal metabolism of the normal group averaged −9 per cent when compared with the Aub-DuBois standards; that of each of the moderately advanced group and of the far advanced group averaged −8 per cent.

3. After breakfast, which was not controlled as to amount or character, the increase in metabolism amounted to 12.4 per cent, 16.2 per cent, and 16.9 per cent respectively for the normals, moderately advanced cases and far advanced cases.

4. The average pulmonary ventilation in the basal condition was 5.69 liters per minute for the normals, 5.57 liters for the moderately advanced cases, and 6.05 liters for the far advanced cases.

5. The increase in ventilation after breakfast was 8.1 per cent, 17.6 per cent, and 18.1 per cent for the three groups.

6. When compared with the increase in oxygen consumption after food, the percentage increase in the ventilation was, in the normals, only two-thirds the percentage increase in the oxygen absorption; whereas, in both tuberculous groups, the percentage increase in ventilation was equal to or slightly in excess of the percentage increase in the oxygen absorption.

CONCLUSIONS

1. In nontoxic, afebrile patients with pulmonary tuberculosis, extent of lesion apparently does not affect basal metabolism. Patients with far advanced pulmonary lesions had a rate which was entirely normal.

2. In nontoxic afebrile patients the pulmonary ventilation, in the basal condition, is normal even in far advanced cases; but when the oxygen requirement is increased the increase in the ventilation in the tuberculous patient is definitely greater than the increase in the normal subject.

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MONOCYTIC LEUCEMIA

With Report of Two Cases

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UNTIL rather recently leucemias have been considered to fall into two main groups—myelogenous and lymphatic.

Since the case reported by Reschad and Schilling[21] in 1913, there have been a number of reports of "monocytic leucemia," and the concept of this third form seems to be gaining acceptance. Most of the reports have been in German, but a recent (1930) review of the literature with report of two additional cases was published by Dameshek[7] in this country. Since this review additional cases have been described by Bock and Wiede[2]; Farley[8]; Weissenback, Martineau et al.[28]; Muenzer[17]; and Lawrence, Josey and Young.[15] References are also found in the recent literature to leucemias in which the type cell is so primitive that it cannot be readily classified. For these the term "stem cell leucemia" has been proposed by Hoff.[14] Cases which might fall in this group have been described also by Ewald,[8] Pinkerton,[20] Feller and Risak,[9] and Bykowa.[4] A case of "hemohistioblastosis" by Gosio[10] was not available for review. There seems to be no clear-cut distinction between some of the cases of "monocytic leucemia" and some of "stem cell leucemia." In the former, very primitive cells often dominate the blood picture. The present report has made use of the term "monocytic leucemia," as it was considered morphologically descriptive.

The first case was followed at the Infirmary of the University of Wisconsin, and is presented with the permission of Dr. William A. Mowry, chief physician of the Student Health Department. A second case was found in the records of the State of Wisconsin General Hospital, and is included by permission of the attending physician, Dr. R. C. Blankinship.

CASE REPORT

Case 1.—H. E. G., a white male university student, aged eighteen, who had appeared at the ambulatory clinic twice early in January, 1930, for treatment of a mild upper respiratory infection, was referred to the Infirmary on January 27, his chief complaint being constant pain and aching in the right thigh, extending from hip to knee, felt both anteriorly and posteriorly, and first noticed the preceding day on arising. No history existed of injury or overuse of the limb. The social background showed a heavy schedule of studies, several hours' work daily as an oil station attendant exposed to extremes of temperature, very irregular meals, and a gradually developing fatigue.

Past medical history: Measles, mumps, whooping cough, chicken pox, scarlet fever, tonsillitis, otitis media, influenza, pneumonia, appendicitis (operated). No recent severe illness.

Family history: Irrelevant.

*From the Department of Clinical Medicine and Student Health, University of Wisconsin.

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Physical examination showed a subsiding upper respiratory infection, with nonpurulent nasal discharge and injection of the pharyngeal and nasal mucous membranes. The tonsils were hypertrophied and cryptic. The skin was ichthyotic. The affected leg was painful enough to produce a moderate limp, but showed no tenderness, deformity, limitation of test movements, or abnormal reflexes.

Preliminary diagnosis: Rhinopharyngitis, chronic tonsillitis, and myositis of the right thigh; ichthyosis.

The patient remained in hospital nine days, receiving rest, heat, salicylates, and other analgesics. X-ray of the pelvis, sacroiliac and hip joints showed nothing suggestive. The temperature ranged from normal to 100° F., averaging 99.4°. The leucocyte counts varied from 9,100 to 7,800, with neutrophilic leucocytes in proportions of from 76 per cent to 91 per cent.

A subsidence of most symptoms and the patient's desire to resume his school work led to discharge on February 5, but he was readmitted four days later. This time his complaint was almost identical with that of the first admission, but the pain was in the left leg instead of in the right. There was now no pain on the right side whatever.

Again a diagnosis of myositis was made, the etiologic factors being regarded as exposure to extremes of temperature in the presence of a low-grade focal infection, probably resident in the hypertrophied tonsils. Further examination, with the discovery of tenderness over the sciatic nerve and definite radiation of the pain from the lower left back well down into the leg, led to a diagnosis of a left sciatic neuritis. Treatment included heat in various forms, galvanism, and anodyne drugs. A search for infective foci resulted in negative roentgenologic findings in teeth, lungs, and paranasal sinuses. Prostatic massage produced no abnormal secretion, the urine showed no abnormalities, blood Wassermann proved negative, and the Mantoux intradermal tuberculin test gave a negative result.

Symptoms gradually decreased from February 12 to February 19, so that the patient's back could be comfortably strapped and walking resumed. At this stage abdominal discomfort, due to what appeared to be mild spastic colitis, called for appropriate therapy, with disappearance of symptoms by February 25. On February 23 occurred a moderate epistaxis, patient saying such bleeding had been common in the past few years.

The patient still complained of some pain, and did not feel well. The tonsils were considered to be a focus of infection, possibly responsible for the myositis, neuritis, and general malaise. After having been postponed one day because of a temperature of 100°, tonsillectomy was performed on February 28; pathologic report on tissue, chronic tonsillitis. Following the operation the tonsillar fossae steadily oozed a small amount of blood, the patient became extremely depressed mentally, and complained of somatic weakness out of all proportion to his observable findings. Eating and sleeping were interfered with, but the throat was not unusually painful at any time.

From the time of his admission the patient's temperature had occasionally reached 99.2° F., but it now began to rise to 100° F. or 101° F. daily. The pulse was dicrotic and often as rapid as 116. The picture was not that of typhoid fever, but a Widal, blood culture, and stool examination were done. All proved negative. Agglutination tests for B. abortus and B. tularense were also negative. Preoperative blood studies had shown: Coagulation time four minutes; hemoglobin 79 per cent; erythrocytes 4,830,000 per c.mm.; leucocytes 4,250 per c.mm.; polymorphonuclear leucocytes 59 per cent. On March 6, the eighth day after the operation, the figures were: Hemoglobin 57 per cent; erythrocytes 3,280,000 per c.mm.; leucocytes 3,900 per c.mm.; polymorphonuclear leucocytes 15 per cent; lymphocytes 81 per cent; large mononuclear leucocytes 1 per cent; myelocytes 1 per cent; eosinophile leucocytes 2 per cent. Two nucleated and an occasional stippled red blood cell were seen. Differential diagnosis lay between an aleucemic type of acute leucemia, an agranulocytic angina, and the leucopenic phase of infectious mononucleosis. The lack of necrosis in the throat, absence of severe pain, and presence of generalized lymphadenopathy (the latter noted first on March 7, 1930) were all against the agranulocytosis, the type of cell against the latter.

Blood smears which had up to this point been examined by a technician were now submitted to Dr. C. H. Bunting, who reported as follows:

"Smear apparently that of an aleucemic phase of leucemia, diagnosis resting upon abnor-

mal character of cells present. Abnormal cells are of extremely primitive character, possibly even primitive mesenchyme cells, and showing marked tendency to formation of pseudoplatelets.

"The striking cell in the smear is a large cell with round or oval nucleus with close packed chromatin masses and definite large nucleolus. The protoplasm of the cell tends to have a clear border with more granular protoplasm about the nucleus. In some cells a few azurophile granules are present in the protoplasm.

"The cells are round or oval, and in parts of the smear where the neutrophiles are so spread as to measure 12 μ in diameter, these cells measure up to 32 x 27 μ, with nucleus of 15 μ in diameter."

From this point on the course was typically that of an acute leucemia. Transfusion of 500 c.c. of whole blood on two occasions produced fleeting relief of symptoms. The daily temperature range was 99° to 101° F., the pulse rate between 88 and 96. On March 14 a violent epistaxis occurred, and three days later the gums were hugely swollen and showed the presence of large numbers of Vincent's spirillae and fusiform bacilli, as the throat had done one month previously. On March 20 the heart developed "gallop" rhythm and a faint systolic murmur was heard in the third and fourth left interspaces. Next day occurred a severe chill, the temperature rising to 103° F., pulse 120, respiration 20, accompanied by a hard, sharp pain in the right chest. Many purpuric spots appeared on the skin of the abdomen and chest, and in the cubital fossae. Chest findings were indicative of congestion generally, and specific enough to lead to a diagnosis of a small infarct of the right upper lobe, with pleurisy and beginning terminal pneumonia. Next morning there was consolidation of the right lower lobe, the heart was tremendously overactive with a loud systolic and diastolic basal murmur transmitted over the whole precordium. Temperature was 103.4° F., pulse 120, respiration 28. Systolic blood pressure was 110, the diastolic not ascertainable. Late that day a loud pleural friction rub became audible at the right base posteriorly. A blood culture made at this time returned negative for all growth. By the morning of March 24 fluid was accumulating in the right pleural cavity. That afternoon a fairly extensive pulmonary hemorrhage took place, succeeded by a large hemorrhage into the bowel. During the night, after a very severe paroxysm of coughing, the patient became extremely weak and could be roused only momentarily. Cheyne-Stokes respirations began shortly thereafter. Copious emesis of dark red blood followed, after which the patient lapsed into deep coma, and death occurred at 7:40 A.M., March 25.

Clinical diagnosis: Acute aleucemic leucemia.

Table I shows blood counts which are of interest.

Autopsy Report, one hour postmortem (from the Department of Pathology, University of Wisconsin Medical School; Dr. C. H. Bunting, Director):

"*Gross Anatomical Diagnosis.*—Generalized lymph node enlargement; hemorrhage into the subcutaneous tissue, mucous membranes, and serous surfaces; hemorrhagic bronchopneumonia; pulmonary infarction; splenic tumor with lymphoid hyperplasia; hydrothorax, left side; indeterminate vegetative endocarditis, aortic and mitral valves; cloudy swelling and fatty change in liver; transfusion incisions, right arm; abdominal scar of old appendectomy; pleural adhesions, right lung; chronic desquamative dermatitis; milky patch on heart; bicuspid aortic valve.

"*Microscopic Report.—Lymph Nodes:* (The same description will apply to all nodes examined, mesenteric, bronchial, superficial.) The architecture of the nodes is almost entirely obscured. A few small clumps of small lymphocytes are scattered through the gland section, usually in the peripheral portion. The rest of the gland is infiltrated with cells chiefly much larger than the small lymphocytes. Many of these are of lymphoblastic character but usually with more extensive protoplasm. In many of these the nuclei are eccentric and lobed as in the monocyte. There are also many cells with extensive protoplasm and sharp vesicular nucleus with distinct nucleolus or nucleoli. Large cells in mitosis are numerous. Scattered among these large cells are intermediate sized and small lymphocytes.

"*Bone Marrow:* Section of the bone marrow of the femur shows a diffuse infiltration with cells of the types described for the lymph nodes. Only scattered neutrophile leucocytes, and an occasional small group of nucleated red cells are seen.

"*Heart:* The epicardium shows a minute hemorrhage, and in certain areas a marked in-

TABLE I

CASE 1

DATE	HB.	R. B. C.	W. B. C.	NEUTR.	LYMPH.		L. M.	MYEL.	EOSIN.	BASO.	LYMPHO-BLASTS	NORMO-BLASTS	PLATE-LETS	COAG. TIME
					S	L								
1/27			9,100	85	14		1							
1/28			8,650	76	24									
1/30			7,800	91	8				1					
2/9			7,050	60	37				3					
2/13	79	4,830,000												
2/20			4,250	59	39		1		1					
2/27														4 min.
3/6			3,900	15	81*		1	1	2					
3/7	57	3,280,000	4,150	23	39				1		37	2		
3/8	59[a]	3,400,000	4,250	30	45				1		24	2	140,000	
3/9			3,950	25	43		1	1			30	1		
3/11	55	3,390,000	4,500	20	61				4		15	2		
3/12	62[a]	3,890,000	4,850	20	53	1		3			23	1		
3/15	60	3,490,000	5,600	5	58			1			36	1	70,000	
3/21	58	3,770,000	7,700	10	51	1	1				38	1		
3/22	59	3,270,000	10,000	7	21			1	1		70			
3/23	54	3,150,000	12,150	4	15						81	1		
3/24	54	3,150,000	15,500	2	28	1					69	1		

*There are very obvious discrepancies in the classification of the various white cells from this point onward, as routine blood examinations were not successful in identifying all the aberrant forms present in the smears. Dr. Bunting's description of mesenchyme cells showed wherein the error had existed, so that these counts are of value only in pointing the general trend of the blood picture. It is probable that practically all of the cells classified as lymphoblasts were "monocytes."

[a]After transfusion.

filtration of its adipose tissue with large mononuclear cells of the types described. Heart fibers in some bundles are definitely hypertrophied. Close to the endocardium one bundle is markedly atrophic. Around the arterioles there is a definite fibrillar increase in connective tissue, the fibrils forming somewhat concentric lamellae, suggestive of rheumatic scars. One cellular Aschoff body is found adjacent to one small vessel.

"*Lung:* Section 1. Section shows congestion and edema, with some infiltration of the alveolar walls in the subpleural part with large cells of the character described.

"Section 2. Shows an area of dense hemorrhage with necrosis of lung framework; on its periphery in one area there are numerous groups of coccoid hematoxylin-staining organisms with considerable leucocytic reaction. In general in the periphery there is a wide zone in which there is an alveolar exudate consisting of a fibrin network, enclosing red blood cells and numerous large and small mononuclear cells with only an occasional polymorphonuclear leucocyte.

"Section 3. Section shows edema, hemorrhage, organized pneumonia, and many areas of interstitial accumulation of leucemic cells. These are especially marked about blood vessels. Many phagocytes in alveoli contain fat vacules.

"*Spleen:* Malpighean corpuscles are small. Pulp is increased in amount due to extensive accumulations of leucemic cells, of the character described.

"*Liver:* Parenchymatous degeneration in atrophic liver cells. Sinusoids dilated and crowded with atypical mononuclear cells, numerous large cells in blood stream in mitosis. V. Kuppfer cells prominent, phagocytic for red cells and lymphocytes and cell pigments.

"*Kidney:* Convoluted tubular epithelium in general granular; much swollen, closing the tubular lumen. In certain tubules minute fat vacuoles are present in the cells. Glomeruli and blood vessels appear normal. Hemorrhage and infiltration with mononuclear cells in adipose tissue.

"*Pancreas, Gall Bladder, Urinary Bladder, and Prostate:* Sections appear normal.

"*Aorta:* Thin-walled but with no definite lesion.

"In general vessels show leucemic clots.

"*Histologic Interpretation.*—Acute leucemia involving lymphoid apparatus and bone marrow, resulting in proliferation of atypical large mononuclear cells and of endothelial cells (primitive reticuloendothelium).

"Old and recent rheumatic lesions in heart. Infected infarcts in lung. Bronchopneumonia. Interstitial leucemic deposits. Leucemic spleen."

DISCUSSION OF CASE 1

There are several points of unusual interest suggested by this case. The patient came under observation early and except for a four-day interval was con-

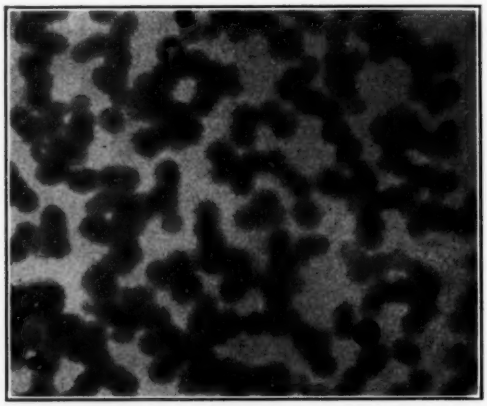

Fig. 1.—Microphotograph of smear from Case 1, March 11, 1930. Magnification ×600. Field containing four cells: (1) Large "endothelial" cell; (2) polymorphonuclear neutrophile leucocyte; (3) small lymphocyte; (4) large lymphocyte or monocytoid cell.

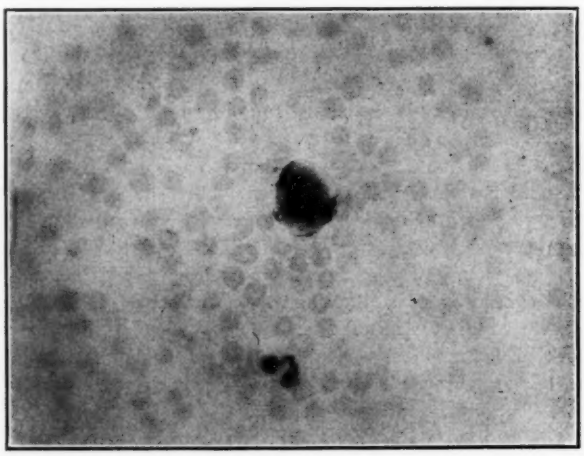

Fig. 2.—Microphotograph of smear from Case 1, March 11, 1930. Magnification ×600. "Endothelial" cell and polymorphonuclear neutrophile leucocyte.

tinuously observed from the onset of symptoms until the fatal termination of the disease. Many students with minor illness who under ordinary circumstances would not be hospitalized are, because of the exigencies of rooming house existence, admitted to the Student Infirmary for care. This was such a case, at the onset. Up to the time of the tonsillectomy the blood picture was essentially normal and there was nothing to suggest the true nature of the disease. There are no characteristic early symptoms of acute leucemia. Frequently vague pains such as this

patient complained of are reported. Sometimes the onset suggests acute rheumatic fever. No satisfactory explanation is offered to account for such pains. Possibly they are due to myositis, neuritis, or arthritis of toxic origin.

The question arises as to what influence if any the tonsillectomy had on the course of the disease, though it probably had none. As noted, the blood picture was essentially normal until the time of operation, but in retrospect we can see that the last white count previous to operation, 4,250 leucocytes, indicated a begin-

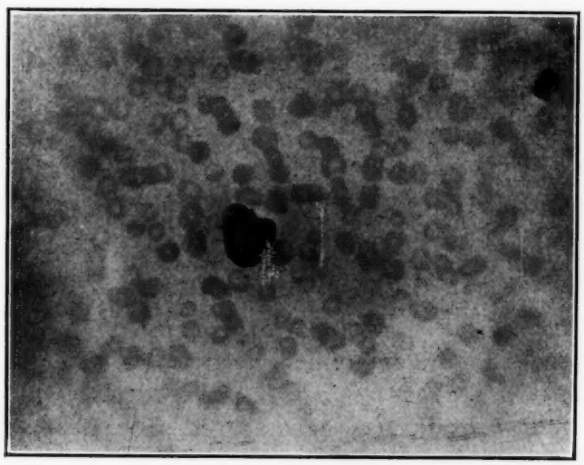

Fig. 3.—Microphotograph of smear from Case 1, March 11, 1930. Magnification ×600. "Endothelial" cell.

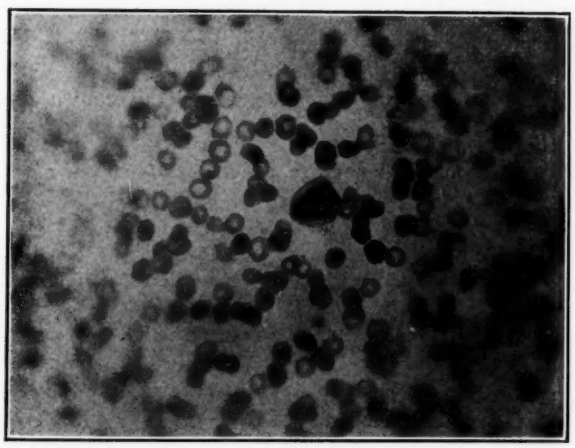

Fig. 4.—Microphotograph of smear from Case 1, March 11, 1930. Magnification ×600. "Endothelial" cell.

ning leucopenia which became more marked shortly after operation. Changes in the differential count, however, were not present until after operation. It is unfortunate that no blood count was made later than one week previous to operation. Of interest in comparison with the present case is one of "excessive hemorrhage after tooth extraction, due to acute leucemia," described by Walmesley.[27] A girl of seventeen bled continuously following tooth extraction until her death on the eighth day. Necropsy revealed acute lymphatic leucemia. An inquest was

held at which it was maintained that, "It was impossible to say that the extraction had accelerated death, and probably it was a coincidence."

The diagnosis of acute leucemia is obvious from the clinical course and the abnormal blood picture. The latter was aleucemic at first but the white count rose to 15,500 before death. The determination of the *type* of leucemia depends upon the identification of the abnormal cells in the blood stream and upon the necropsy findings.

The abnormal cells are not of the type ordinarily found in either lymphatic or myeloid leucemia. The routine counts classify them as "lymphoblasts," but according to Dr. Bunting's report they should be considered extremely primitive cells. The large numbers of small lymphocytes in the smears are in marked contrast to the usual findings in acute lymphatic leucemia, where the leucocytes are almost exclusively large lymphatic elements. The majority of the neutrophile leucocytes show nonsegmented nuclei, usually regarded as indicating immaturity, but transition stages are not seen between them and the very large leucemic cells. Besides the "striking cells" described by Dr. Bunting, there are many cells varying in size from the largest of those just mentioned down to about 14 by 16 μ, similar in appearance except that the protoplasm is more deeply basophile and completely filled with close-set granules instead of having a clear border. Many of these cells have nucleoli. Most of them are round or oval in shape but some are irregular in outline, and many are polygonal, very comparable in shape to ordinary squamous epithelial cells. (The latter may be distorted.) The smaller of these cells approach the morphology of ordinary monocytes. The absence from the routine counts of cells classed as large mononuclears indicates that entirely characteristic mature monocytes are rare. Sections of the spleen, liver, bone marrow, and lymph nodes show infiltration with cells similar to those found in the blood smears. (Figs. 1-4 are microphotographs showing characteristic cells from a smear made March 11, 1930.)

Case 2.—G. W., a white female, housewife, aged thirty-four, was admitted to the Wisconsin General Hospital May 29, 1925, her chief complaint being pain in the arms. Two days prior to admission she had experienced pain in the hips and back which shifted to the arms a few hours later. This was severe enough to prevent sleep, and although at first relieved by aspirin was now apparently not amenable to such medication. The pains were sharp and shooting, changing to soreness after a few hours.

History showed that there had been transient stiffness and some pain in the arms for two years previously. Lately these limbs had felt stiff most of the time. Previous attacks had been well controlled by aspirin. She complained of a few dark discolorations on the skin, resembling bruises but without history of injury. There was a pain in the right chest resembling that in the arms, somewhat aggravated by deep breathing or coughing. History included chronic constipation, and some leucorrhea and menorrhagia.

Past medical history: Measles, chicken pox, whooping cough as a child. Mild influenza in 1920. Many attacks of tonsillitis. Two attacks of quinsy. Tonsillectomy in 1923. There had been two normal deliveries, no miscarriages; the last baby was nine months old at the time of the patient's admission.

Social history and family history: Irrelevant.

Physical examination showed gingivitis, slight inguinal adenopathy, ecchymotic areas on the limbs, cervical laceration and erosion, third degree uterine retroversion. The diagnosis was bilateral brachial neuritis, probably secondary to chronic endocervicitis; gingivitis. Additional history on June 16, 1925, told of mild attacks of nausea for years. There were sudden seizures

of nausea and cramplike pain in the epigastrium, usually coming on while the patient was eating. Symptoms were relieved by induced vomiting. Impression: Chronic cholecystitis, possibly with cholelithiasis.

X-rays of the paranasal sinuses and left shoulder were negative; of the teeth showed an impacted unerupted upper left third molar with slight periapical involvement of the upper right second bicuspid. Roentgenologic studies of the gastrointestinal tract showed nothing abnormal except a moderately delayed emptying time for the stomach due to hypomotility. Fractional gastric analysis was done and showed an achylia and the constant presence of occult blood. Other laboratory examinations included urine, containing traces of albumin and traces of glucose, a negative blood Wassermann, normal blood chemistry, normal coagulation time and fragility test, and blood counts within limits of ordinary observation.

Treatment consisted of local applications to the gums, and electric cauterization of the lacerated cervix together with pessary support of the retroverted uterus. She was discharged from hospital on June 19, 1925. Patient was readmitted to hospital one week later for further study, as it was felt that the original diagnosis had not been complete. She had gained three or four pounds in weight, but the painful symptoms had recurred.

Reexamination of the stomach and duodenum roentgenologically gave normal findings. Pelvic examination disclosed excellent healing in the cervix, and the pelvic condition generally was considered normal. Spontaneous slight bleeding from the gums continued, and smears showed presence of Entameba gingivalis. Fresh stools showed flagellates. There was now a prolonged bleeding time, and repeated blood counts showed a gradual dropping in the number of leucocytes as well as in the erythrocytes and hemoglobin. The most significant feature of the blood picture was a continued lowering of the percentage of granular leucocytes and the blood platelets. The temperature, which had been normal for the first four days, began to show daily variations between 99° and 103°, of a septic type.

The diagnosis now arrived at was that of a leucemia of the aleucemic type. The general condition became progressively worse until July 28, when the first transfusion was given with definite improvement for a period of several days. By August 23 pain was returning, being now present in the left side of the face, the chest, and the lower back and thighs. On September 5 chest examination showed marked elevation of the right diaphragm, thought to be due to palpable enlargement of the liver; confirmed by x-ray. Transfusions of citrated blood were given on three subsequent occasions, the first two resulting in temporary improvement, the third being apparently without effect. Horse serum was also given, with temporary benefit. Marked cervical and inguinal adenopathy had been present as early as September 30. From October 21 the patient declined very rapidly. Pain in various areas became steadily worse, and bleeding from the gums increased. There was considerble vomiting. Semiconsciousness supervened by October 25 and the patient died early the following morning.

Autopsy Report, one hour postmortem (from the Department of Pathology, University of Wisconsin Medical School; Dr. C. H. Bunting, Director):

"*Gross Anatomical Diagnosis.*—Lymphocytic leucemia; exhaustion of bone marrow; anemia; fatty degeneration of parenchymatous organs; zonal necrosis of liver; splenic tumor; auricular thrombus; embolism of pulmonary artery; infected infarct right lung; pneumonia; cholelithiasis.

"*Microscopic Report.—Lymph Nodes:* The general architecture of gland has disappeared. Small groups of lymphocytes suggesting the Malpighian corpuscles of the spleen are grouped about the arterioles in the region of the former germinal centers. The rest of the gland is diffusely infiltrated with large pale cells. These vary in size and character. Some evidently belong in the primitive stages of the lymphoid series, while many are definitely of an endothelial character with abundant protoplasm and large vesicular nucleus with definite nucleolus. Mitotic figures among these cells are numerous.

"*Spleen:* The Malpighian corpuscles are small, consisting in the main of small lymphoid cells. The pulp of the spleen and its sinuses are crowded with cells of the character of those described for the lymph nodes. Considerable hemosiderin is found in phagocytes in the pulp.

"*Rib Marrow:* The rib marrow shows almost complete exhaustion of hemopoietic centers, and atrophy of fat. The vascular architecture is well marked, also the reticular structure of

TABLE II*

CASE 2

DATE	HB.	RED BLOOD CELLS	WHITE BLOOD CELLS	NEUTR.	S. LYMPH.	L. LYMPH.	INT.	L. M.	MYE.	TRANS.	EOSIN.	BASO.	LYM-PHO-BLASTS	NORMO-BLASTS	UNNAMED MONO. CELLS (PATH.)	DEGEN. CHANGES IN R. B. C.	PLATELETS	COAG. TIME	BLEED. TIME
5/30	82	4,430,000	9,000	56.5	29	2.6		10.5		0.4	0.7	0.3						7½ min.	
6/8	76	4,420,000	6,750	58	37	2				2	1								
6/18	62	4,370,000	4,900	49	38	9				4									
6/30	55	4,055,000	3,500	39.2	41	17		2			0.4	0.4		2		Mod.	Decreased		25 min.
7/10	55	3,280,000	3,600	46.2	32.8	4.6		1.2	1	0.2	1.6			1	12.4	+	217,500		
7/29	43	2,960,000	1,300	26.2	53.6	6		4.2	1		2				7	+			7 min.
8/17	50	2,610,000	3,850											3		Mod.			2½ min.
8/26	53	3,660,000	4,200										4	4			Decreased		7 min.
9/14	40	3,350,000	5,900	18.6	71.8	5.3			1.5	1.2	0.2	0.2		10	1.2	Mod.	Decreased		17 min.
9/28	20	2,065,000	9,850	9	60.5	30.5								4		Mod.	Decreased		
10/6	23	1,940,000	15,650	3.8	25	26	44.6	0.2									Much decr.		62 min.
10/15	15	1,492,000	20,600	2.2	25.4	15.8	48.6								8	+			45 min.

*As in the first case, these blood counts are to be regarded only as a rough indication of the general blood picture. Smears of the blood were examined by Dr. C. H. Bunting and found to contain large atypical cells of the same type described by him in Case 1.

the marrow. There is some edema, areas of hemorrhage with fibroblastic proliferation, also a few perivascular collections of large cells of the type described. There are also scattered lymphocytes in the marrow.

"*Femoral Marrow:* Similar to rib marrow, with possibly larger perivascular collections of primitive cells.

"*Heart:* Section shows congestion and edema of heart wall, slight atrophy of muscle fibers, with hydropic degeneration.

"*Heart Thrombus:* Shows dense almost hyaline platelet columns, with heavy fibrin border and well marked intervening fibrin network, containing chiefly red blood cells and atypical leucocyte forms. The usual neutrophile border to the platelet columns lacking.

"*Lung:* Small area of necrosis of lung tissue with many colonies of gram-staining cocci in it. Surrounding areas of pneumonia, hemorrhage, and edema. Pneumonic exudate shows well marked fibrinous network with a high proportion of neutrophile leucocytes in some areas, and in others chiefly the large mononuclear type of cell.

"*Liver:* Sinuses of liver lobule dilated and containing many mononuclear leucocytes. General atrophy of liver cells of central and mid-zone of lobule, with fatty degeneration and some necrosis. Cells of peripheral zone are somewhat swollen, granular.

"*Kidney:* Tubules of cortex much dilated and containing heavy albuminous precipitate. Cells of tubules swollen, granular and hydropic. Capsules of glomeruli are dilated, albuminous precipitate. In the boundary zone and medulla the interstitial tissue shows numerous collections of cells of great variety. There are megalokaryocytes, neutrophile and eosinophile leucocytes and myelocytes, and nucleated red cells. Suggesting a myeloid transformation.

"*Pancreas:* Atrophy, lipomatosis, well marked interacinar pancreatitis; islands generally, hypertrophic.

"*Aorta:* Atrophy and degeneration of media. Atheromatous nodule in intima.

"*Uterus and Ovaries:* Senile atrophy."

DISCUSSION OF CASE 2

There are several points of similarity with Case 1. This patient also was observed from the onset of symptoms until the fatal termination. There was an onset with vague pains leading to a diagnosis of neuritis, and a focus of infection was considered to be the etiologic factor. At first there was nothing to suggest the true nature of the case, except the presence of gingivitis and ecchymotic areas. The blood counts during the first admission were essentially normal. Here, as in the first case, there was gradual decrease in the granular leucocytes with increase in cells classified as lymphocytes. The total number of leucocytes dropped to a low level of 1,300 per c.mm. but toward the end rose to 20,600. The course of the disease was somewhat less acute than in the first case, extending over a period of five months as contrasted with two months in the former. Here, as in the first case, a typical clinical and blood picture of leucemia gradually developed. While the gross anatomical diagnosis was lymphocytic leucemia, microscopic examination showed the type cells to be very primitive in character. Some of them evidently belonged in the primitive stages of the lymphoid series but many were definitely of an "endothelial" character.

GENERAL DISCUSSION

These cases of leucemia are presented for their clinical interest in that both were followed from the onset of symptoms to a fatal termination and necropsy, and also because of the very unusual blood pictures. Although, unfortunately, the clinical study throws no light on the etiology of this disease, nor the pathologic study on the origin of the abnormal blood cells, it is hoped that the addition to the

literature of two more cases of an unusual type of leucemia may be of some value as an addition to the data available for study.

The exact classification of these cases in relation to others previously published is difficult. They appear to differ somewhat from the cases reviewed by Dameshek,[7] and the more recent cases of monocytic leucemia in the absence from the blood stream of large numbers of typical monocytes. They perhaps are allied to the rather heterogeneous group of primitive-cell leucemias described under various names by Bykowa,[4] Feller and Risak,[9] Hittmair,[12, 13] Hoff,[14] and others.

Most of the reports of monocytic leucemia have favored a reticuloendothelial origin for the monocyte, and several authors have described transitions from reticuloendothelial cells through free histocytes to mature monocytes. In using the term monocytic leucemia here, there is no intent to advocate any particular theory of origin of the characteristic abnormal cells or of monocytes in general.

A review of the varied hypotheses gleaned from a review of the literature on monocytic leucemia and the monocytes may be of interest.

There seems to be general agreement among hematologists that in postnatal life undifferentiated mesenchymal cells exist which may under certain circumstances give rise to all forms of blood cells. By the polyphyletists these are considered to be the only pluripotential "stem cells." The monophyletists believe in a hemocytoblast, a derivative of the primitive mesenchyme, which *normally* gives rise to all forms of blood cells. It would appear that there is considerable confusion of terms and ideas relative to the stem cells. By many writers the reticuloendothelial system is regarded as synonymous with primitive mesenchyme. Maximow,[16] on the contrary, ascribes very limited blood forming powers to the reticuloendothelial system and describes other "undifferentiated mesenchymal cells." He says, "In the reticulum of the lymphoid tissue of vitally stained animals, the large phagocytic reticular cells which store the dye, the typical histocytes, can be distinguished from small, pale elements of the syncytium, which contains few or no inclusions and are especially distinct in the germ centers." He claims that these syncitial elements, found in the common connective tissue as well as in that of the blood forming organs, are "fixed undifferentiated cells which keep their embryonic mesenchymal potencies."

To illustrate the confusion of terms and the lack of distinction between reticuloendothelial cells and undifferentiated mesenchyme the following may be cited: Pinkerton[20] uses synonymously the terms "adventitial cells," "clasmatocytes," "polyblasts," and "hematohistoblasts," and considers them "*undifferentiated mesenchymal elements.*" Dameshek[7] names as constituting the "*reticuloendothelial system*" cells variously called "clasmatocytes," "macrophages," "resting-wandering cells," and "histocytes." He later uses as synonymous "hemocytoblast," "hemohistoblast," "histocyte," and "Stammzell." Richter[22] says of the hemohistoblast of Ferrata, "Morphologically the cell has been identified with the group of tissue cells variously known as 'clasmatocytes' (Ranvier), 'resting-wandering cells' (Maximow), 'adventitial cells' (Marchand), '*reticuloendothelial system*' (Aschoff)." He agrees with Ferrata in considering the hemohistoblasts "*veri elementi mesenchimatici embriali.*" Maximow[16] considers "lymphocyte" and "hemocytoblast" synonymous. He says, "The so-called unitarian theory—

claims that there is only one stem cell and that the common small lymphocyte of the blood."

Numerous cases called, from their pathologic picture, leucemic reticuloendothelioses have appeared in the literature during the present century. Many of these have shown in the blood stream, in addition to histocytes, large numbers of cells described as typical monocytes. By those believing in the reticuloendothelial origin of the monocyte it is felt that the term leucemic reticuloendotheliosis should be applied to all monocytic leucemias. The term "histocyte" is used with such a variety of connotations that it leads to some confusion. At times it is used as synonymous with reticuloendothelium, again it serves to designate only *free* reticuloendothelial elements either in the blood stream or in the tissues. By many the term is used to mean those mononucleated cells in the blood stream which they regard as immature monocytes, transitional forms between fixed reticuloendothelial cells and mature monocytes. The histocyte is thought by them to bear the same relation to the monocyte as the myelocyte to the mature leucocyte. Dameshek's[6] recent paper on the histocyte mentions this point of view. The essential rôle of the reticuloendothelium in leucemias in general is discussed by Bykowa[4] in a recent article (1931). He quotes from Hoff, "The reticuloendothelial system under pathologic conditions can differentiate in various directions according to the type of stimulus and lead to myeloid, lymphoid, or monocytoid leucemias and their combinations." Two cases of Hittmair (V and VI)[12, 13] serve to illustrate this fact. In Case VI at one period myeloid cells predominated; at another, lymphatic; and at a third period, more primitive cells. Case V was a chronic case treated by x-ray which showed ten exacerbations. At one time "lymphoidocytes" dominated the picture, at another reticuloendothelial monocytes, and again cells of the myeloid series. The case of Wyschegorodzewa[29] cited by Dameshek as one of monocytic leucemia showed at the end a change from a monocytic to a myeloid character. The author comments that this change speaks for the possibility of formation of myeloid elements from monocytes. It is conceivable, however, that early in the disease the reticuloendothelium was stimulated to the production of monocytes and toward the end, for some reason, to the production of myeloid cells.

The question of the origin of the monocyte is still much debated. Besides the theory of direct reticuloendothelial origin, Bloom[1] cites other theories: (1) origin from myeloid cells, (2) origin from lymphatic cells, and (3) origin from ordinary vascular endothelium. Cunningham, Sabin, and Doan[5] suggest an origin from a "primitive blood cell" derived in turn from a reticulum cell. Early writers did not distinguish between monocytes and free histocytes. Sabin, Doan, and Cunningham[23] in 1925 distinguished in the tissues by the supravital technic two types of phagocytic cells which they called clasmatocytes (the histocytes of other authors) and monocytes. There seems to be very general agreement that the histocytes (clasmatocytes) are probably direct derivatives of reticuloendothelium. In fact, one frequently finds the terms reticuloendothelial system and histocytic system used interchangeably. Views as to the origin of the other large mononuclear phagocytes usually referred to as monocytes remain divided as indicated above.

The reasoning in practically all instances is that, under various circumstances, all transitional stages can be found between the monocyte and the cell from which the particular observer believes it to be derived. We may cite, e. g., the following observations in favor of the several theories:

Myeloid Origin: Piney[19] has seen all transitions between myeloblasts and monocytes. He describes a "promonocyte" which is sometimes impossible of differentiation from a myeloblast.

Ehrlich believed he saw transitions from large mononuclears, by way of the transitional cells, to neutrophile granular leucocytes. (This observation is now generally conceded to be incorrect. Naegeli, the chief follower of Ehrlich in supporting the myeloid origin of the monocytes, bases his opinion on the *oxydase reaction.*)

Lymphatic Origin: Weidenreich and others (according to Bloom[1]) have described a morphologic similarity between lymphocytes and monocytes with transitional stages.

Bloom, himself, using various stains including the supravital, saw in smears of the blood, and in smears and sections of the blood-forming organs, all transitions from lymphocytes to monocytes. His observations were made on animals in which monocytosis had been induced by injections of B. monocytogenes or colloidal substances.

"Primitive Cell Origin": Sabin, Doan, and Cunningham[23] state that splenic puncture shows all steps in the origin of the monocyte from the primitive blood cell. They have seen, also, in tuberculous organs very young monocytes touching a primitive cell on the one hand, and mature monocytes on the other.

Histocytic Origin: Schilling believes (Bloom[1]) that in endocarditis lenta and monocytic leucemia he has seen transitions between histocytes and monocytes.

Swirtschewskaja[26] describes a case of "reticuloendotheliosis" in which the blood contained typical monocytes, and in which after necropsy, sections of the spleen showed all transitions from the endothelial cells lining the splenic sinuses to free cells of the monocyte type.

Dameshek[7] notes that a direct association between proliferative reticuloendothelial cells and the leucemic cells has been described in monocytic leucemia by several authors.

It would seem either that the monocytes have a multiple origin or that the method of determining origin of a cell by morphologic similarity is unreliable.

A new method of attack was used by Silberberg[25] which seems definitely to disprove an exclusive myelocytic origin of the monocytes. By poisoning with benzol he obtained animals in which the circulating blood was free of granular cells. Monocytes were still present in the circulation. Tissue cultures of the spleen and bone marrow from these animals showed histocytes and monocytes with all transitions, as well as lymphocytes, but no cells of the myeloid series. No mention is made of transitions from lymphocytes to monocytes, so that this work favors the histocytic origin of the monocyte as opposed to the lymphatic, although it does not definitely exclude the latter. In cultures of spleen and bone marrow from leucocyte-free animals vitally stained with carmine, he saw some carmine in the monocytes as well as in the histocytes. One of Bloom's[1] arguments against the histocytic theory was that none of the monocytes in his vitally stained animals took the vital dye. A possible weakness in these experiments is the identification of monocytes by the supravital stain. Hall[11] has shown in his review of the literature on supravital staining that this method cannot be relied upon for the identification of monocytes "because there are too many other types of rosette cells in the tissues that may be confused with monocytes."

The oxydase reaction, usually considered a criterion of myeloid cells, is apparently of little aid in the solution of the monocyte question. Monocytes generally are described as oxydase positive, but Ordway and Gorham[18] in the Oxford Monographs state that "a small percentage, perhaps 5 per cent, react negatively." Schilling[24] says they are either negative or faintly positive to the oxydase stain. Dameshek[7] states that the oxydase reaction is faintly positive. Uyeyonahara (1930), according to Hall,[11] supports the theory of multiple origin of the monocytes. He reports a classification of hematogenous monocytes on a basis of peroxy-

dase reaction and storage of carmine colloidal dye: Monocytes showing either a negative or positive peroxydase reaction and containing carmine granules are derived from histocytes; those possessing peroxydase granules but no carmine granules are of myeloid origin; and those containing neither carmine nor peroxydase granules are of lymphatic origin. His method consists of incubating citrated human blood at body temperature and exposing it for varying intervals of time to a solution of carmine. Dry smear preparations are made and stained for peroxydase granules. Hall[11] considers this classification questionable, for Downey offers convincing evidence to show that dye storage cannot be used as a criterion for separation of the various cell elements in the blood and tissues. Bock and Wiede[2] feel that it is likely that even yet diverse forms are grouped together under the name of monocytes.

SUMMARY

Two cases of leucemia, duration two months and five months respectively, are reported. Certain similarities of early symptomatology are noted, as well as corresponding difficulty of recognition of aberrant cells in the blood smears. In each instance, the predominating cell was too primitive to admit of hard and fast classification. Other cases, apparently parallel in most respects, are mentioned. A brief review of the literature concerned with the rôle of the reticuloendothelium in leucemia is included, together with the views of various authors on the origin of the monocyte.

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LABORATORY METHODS

FURTHER STUDIES ON A COMPARISON OF THE HUDDLESON SLIDE TEST WITH THE MACROSCOPIC TUBE TEST IN UNDULANT FEVER*

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IN A previous article[1] the authors demonstrated that the Huddleson "Rapid Method" for determining the presence of agglutinins of the Brucella group in the blood sera of human beings was slightly more sensitive and specific than the macroscopic tube test used. The rapidity and ease with which the Huddleson test may be carried out made it an excellent test for public health laboratories where speed and accuracy in reporting results to physicians are important factors.

Since our previously published series included only 156 specimens it seemed desirable to continue the investigation. For the past year a total of 763 examinations for the presence of agglutinins for the Brucella group have been made.

METHODS

The first 120 sera were examined by three methods, two macroscopic tube tests and the Huddleson method. One of the tube tests was done for us by Dr. W. N. Plastridge at the Connecticut (Storrs) Agricultural Experiment Station and the method has been reported previously.[1] The Huddleson "Rapid Method" has been described in a number of earlier publications.[2, 3, 4] The third test—a macroscopic tube test—is described below as the "Conn." test.

The antigen employed in the Conn. test noted above was made from four strains of Brucella abortus, coming from different sections of the country, whose agglutinability had been carefully checked. The strains were grown for twenty-four hours on 1 per cent glycerine Fairchild's agar and transplants were made from these onto plain Fairchild's agar and incubated forty-eight hours at 37° C. The growth was harvested with a small amount of physiologic salt solution containing 0.4 per cent phenol and diluted for test purposes to a reading of 7.8 to 8 on the Gates[5] apparatus.

Test tubes, 75 by 10 mm., were used; 0.5 c.c. of diluted serum being placed in each tube, the dilutions ranging from 1:25 to 1:3000 in a regular sequence and 0.5 c.c. of antigen finally being added to each. The tests were incubated in a 56° C. water-bath for six hours followed by overnight in the refrigerator at 8° C. The degree of clumping was estimated and tabulated at +, ++, +++ or ++++ representing respectively 25 per cent, 50 per cent, 75 per cent and 100 per cent agglutination.

RESULTS

An arbitrary classification has been in use for several years for the routine reporting of results.

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Specimens showing titers of 2+, 3+, or 4+ in any dilution or dilutions including 1:300 and above are classed as "positive." Specimens are classified as "questionable" if they show any reaction either above or below 1:300 when they fail to fall in the positive class. All other examinations are reported as negative. This standard was purely arbitrary but gave us a definite basis for the reporting of specimens.

As previously reported,[1] in our experience active clinical cases of undulant fever rarely give a positive agglutination reaction that is not above a titer of 1:100, although a positive reaction in a titer of 1:100 is considered diagnostic by some.

The results obtained from all three tests on 120 sera are given in Table I.

TABLE I

RESULTS OF AGGLUTINATION BY THREE METHODS ON 120 SERUMS

METHOD	POSITIVES	QUESTIONABLES	NEGATIVES	TOTALS
*Storrs	10	30	80	120
Huddleson	9	9	102	120
*Conn.	9	5	106	120

*Macroscopic tube method.

It is at once apparent there is close agreement as to "positives" by the three methods. The Storrs method on the basis of our arbitrary classification gave 10 positives as against 9 by the other two methods. The physician in charge of the case found positive by the Storrs method but questionable by the other two methods, stated that the patient's history failed to show symptoms of undulant fever.

The questionable reactions obtained were considerably greater with the Storrs method than with the other two (note Table I), the former method giving 30 questionable results as against 5 and 9 respectively for the Conn. and Huddleson methods.

Table II gives the reactions obtained with 763 serum examinations for the year 1931 with the Conn. and Huddleson Rapid methods.

It will be noted that the Conn. test (macroscopic tube) gave six more positives than the Huddleson on the basis of our standards for laboratory diagnosis. In each of these six serums, however, the Huddleson did show some reaction at least in the 1:100 dilution which, as has been pointed out, is considered diagnostic by some laboratories. Further, two of these serums were repeated tests which previously had shown an agglutination in the 1:3000 dilution with the Huddleson method. Further it will be noted (Table II) that the tube test showed 45 more questionable reactions than did the Huddleson method, indicating a definite difference in specificity of the two tests.

In order to obtain more accurately the value of the tests used for the diagnosis of undulant fever, questionnaires were sent to all physicians with laboratory-diagnosed positive cases. In Table III these results are summarized. It will be

TABLE II
RESULTS OF AGGLUTINATION WITH CONN. AND HUDDLESON METHODS ON 763 SERUMS

METHOD	POSITIVES	NEGATIVES	QUESTIONABLES	TOTALS
Huddleson	*59	645	*59	763
Conn.	65	594	104	763

*Note: There were eleven repeated tests. In two of these the Huddleson had dropped from a titer of 3,000 to 100 and hence fall in questionable group.

noted that there were 31 cases of undulant fever so diagnosed by physicians which gave positive agglutinin reactions. There were eleven tests repeated in the series of 65 laboratory positive cases, 22 positive by agglutination reported negative by physicians and one case undiagnosed. In this undiagnosed case and in those cases numbered 47,467 through 69,261, although a diagnosis of undulant fever was not made in most instances, definite fever symptoms developed during the illness. In specimen 56,846, in which no diagnosis was made, agglutinins for the Brucella group were present in the 1:3000 dilution and the clinical evidence indicated fever symptoms suggestive of typhoid, yet there were no agglutinins for Eberthella typhi nor did rose spots develop. In one case listed in this group of undiagnosed cases, 46,532, the patient was said to present no symptoms of any disease and appeared well; yet agglutinins for the Brucella group were present in a dilution of 1:3000. Such high titered agglutinins seem to us necessarily to indicate a Brucella infection in spite of the absence of classical clinical symptoms. It will be noted in Table III that the age group in which the diagnosis was most often given definitely as undulant fever was in the adult group, whereas cases in children and young adults were more often called either mild, atypical, or not undulant fever.

Specimen 69,261 (child, aged three and one-half years, Table III) was not clinically diagnosed as undulant fever. The titer by the Huddleson method was 1:100 and by the macroscopic 1:300. This was reported as a positive case by these Laboratories inasmuch as it fell within the range of our arbitrary standards. The physician reported an unexplained fever: "Fever 99° to 100.8° for several weeks with a moderate pharyngitis and very slight cervical adenitis as the only physical signs." The agglutinin titers were comparatively low in this particular case but seem to us sufficiently high to raise the question whether or not a mild Brucella infection did not exist. In addition, the physician notes that this child had drunk considerable quantities of raw milk.

Sasano[6] and his co-workers report that "the examination of serums from 1000 persons revealed a positive complement fixation for Brucella abortus in 96 and a positive agglutination in 78. In only five cases was the diagnosis of undulant fever made. In each of these five cases complement fixation was positive, and agglutination was present in titers of from 1:135 to 1:200 at some time during the illness." Sasano further notes that a diagnosis of undulant fever should not be made on the basis of agglutinations in dilutions of 1:15 or 1:45 in the absence of a positive blood culture. A study of the case mentioned in the preceding paragraph and others to be discussed later indicates that extremely mild cases of

TABLE III

ANALYSIS OF SIXTY-FIVE LABORATORY-POSITIVE CASES OF BRUCELLA INFECTION

SPECIMEN NUMBER	PHYSICIAN'S DIAGNOSIS	LABORATORY TESTS		PATIENT'S AGE	REMARKS
		HUDDLESON	MACROSCOPIC		
85080	U. F.	3000	3000	None given	
11306	U. F.	2000	300	29	
10879	U. F.	3000	1500	51	
11530	U. F.	3000	3000	23	
521	U. F.	3000	3000	None given	
73733	U. F.	3000	3000	45	
3643	U. F.	3000	3000	46	
88241	U. F.	3000	3000	32	
35593	U. F.	3000	3000	35	
59748	U. F.	3000	1500	None given	
42476	U. F.	3000	1000	57	
35944	*U. F.	3000	3000	57	Repeat on 42476
5115	U. F.	3000	3000	37	
6682	U. F.	3000	3000	52	
27287	U. F.	300	300	45	
39596	U. F.	3000	3000	None given	
71892	U. F.	3000	3000	60	
7365	U. F.	3000	1500	17	
64440	U. F.	1000	750	14	
77986	U. F.	3000	3000	43	
7800	U. F.	3000	300	29	
84827	*U. F.	3000	1500	29	Repeat on 7800
76557	*U. F.	100	200	29	Repeat on 7800
26918	U. F.	3000	3000	20	
37488	U. F.	3000	1500	45	
32510	*U. F.	1000	750	45	Repeat on 37488
30782	U. F.	3000	3000	35	
13831	U. F.	3000	1000	21	
47467	–	3000	3000	45	Fever symptoms
48374	–	3000	3000	16	Fever symptoms
44788	–	3000	3000	16	Fever symptoms
15698	–	750	300	None given	Habitual abortion
74696	–	3000	750	61	Fever symptoms
50240	–	3000	3000	None given	Diagnosis uncertain; moderate fever
46532	–	3000	3000	61	Patient well; no symptoms
56846	None made	3000	3000	62	Fever symptoms; suggestive of typhoid
69261	–	100	300	3½	Unexplained fever
79769	U. F.	3000	2000	None given	
6686	U. F.	3000	3000	18	
27291	*U. F.	100	300	18	Repeat 6686 (two months later)
1685	U. F.	3000	1500	14	
6854	U. F.	3000	3000	16	Mild case
6856	U. F.	100	750	14	Mild case
21617	U. F.	2000	300	17	Mild case
75066	U. F.	3000	300	14	Mild case
10117	*U. F.	3000	1500	14	Repeat on 75066
22700	–	750	1000	14	N. S. No illness
25668	–	1500	750	14	N. S. No illness
6469	–	3000	1500	17	N. S. Coryza and fever (recovered)
22698	–	1500	1000	18	N. S. Bronchitis

Note: U. F. = Undulant fever.
* = Repeat tests
N. S. = No symptoms of undulant fever. A diagnosis of undulant fever was not made.

TABLE III (CONTINUED)

SPECIMEN NUMBER	PHYSICIAN'S DIAGNOSIS	LABORATORY TESTS		PATIENT'S AGE	REMARKS
		HUDDLESON	MACROSCOPIC		
25666	–	3000	300	16	N. S. No illness
27289	–	1500	300	14	N. S. No illness
22710	–	2000	2000	18	N. S. After chickenpox
2191	–	3000	3000	17	N. S. Six tests on patient 2191 over a period of ten months. Physician states at no time were symptoms of undulant fever present. Upper respiratory infection and mumps during year
21874	*–	3000	2000		
28226	*–	1000	1000		
42279	*–	3000	3000		
66667	*–	1000	750		
76022	*–	2000	500		
6684	–	50	300	19	N. S. Upper respiratory infection (3 days)
25242	–	Insufficient	500	15	N. S. No illness
31392	–	Insufficient	300	17	N. S. Upper respiratory infection (2 days) mild otitis media
2494	–	25	500	18	N. S. Upper respiratory infection—fever—coryza
26914	–	100	200	17	N. S. No illness previous to test
27572	–	100	500	15	N. S. Upper respiratory infection (mild)

undulant fever may exist and because of the vagueness of the symptoms not be recognized as such. Borman notes (personal communication) a case which would not have been recognized except for a continued series of daily temperatures. He reports as follows:

"Patient, a boy aged nine years, had been suffering from a middle ear infection of undetermined etiology. After subsidence of symptoms his mother, a trained nurse, continued to take his temperature daily. It was noted that his temperature with occasional intermissions ran consistently above normal (½° to 1°), especially after hard play. Boy fatigued easily. No other symptoms. The abnormal temperature was checked several times by the physician's thermometer and the mother's thermometer was checked by taking the temperature of normal individuals. This condition remained for the better part of a year. The ear condition was pronounced completely cured by two physicians. Physical examination was negative for any abnormalities to which this condition might be ascribed. X-ray findings were negative for tuberculosis. Urine was repeatedly normal.

"A macroscopic agglutination test for undulant fever was made which was positive (++++) in 1:2560 dilution. Based on this, a diagnosis of undulant fever, mild in nature, was made by the physician.

"History of continued ingestion of raw milk.

"When last heard of, one year after making test, boy was developing normally but continued to run slight temperature at intermittent periods."

Cases such as the above would be missed entirely under ordinary conditions.* The rise in temperature was slight and could be ascribed to the middle-ear in-

*Since this investigation was completed a similar case was encountered.
Case P.—Boy, aged nine. History somewhat like that of grippe. Afternoon temperature 100-103°; morning normal to 100°. Physical examination negative except for loss of weight. No pain. No glandular involvement. Only symptoms apparent, temperature, and lassitude. White cell count 10,000. Widal, Weil Felix, Kahn and Wassermann tests negative. The Huddleson rapid method showed complete agglutination in the 1:6000 dilution.

fection during the illness. These cases indicate to us that very mild cases of undulant fever may exist particularly in children and unless followed closely would not exemplify themselves in observable symptoms.

Twenty-seven specimens (6,686 through 27,572) were received from one physician* who was investigating an outbreak of undulant fever in one of the preparatory schools for boys in the State. Inasmuch as he was in charge of this group of boys he had an excellent opportunity to study the series of cases that developed. He has kept careful case records of the patients that showed both the typical and atypical types of undulant fever. On the group of specimens reported positive by these Laboratories and in which the patients showed no clinical symptoms of undulant fever, he has taken temperatures carefully over a period of weeks and recorded detailed case histories. A large number of specimens have been checked in other laboratories, and the physician has checked in his own laboratory the agglutinin titers particularly in cases where clinical symptoms were not comparable to the serologic results. Only in one case was a definite discrepancy noted and in that instance it appeared to be due to a laboratory error.

Following the development of two cases of undulant fever (6,686 and 1,685), an investigation of the condition of the herd from which the milk was obtained was made by the physician. He found that one of the cows in the herd had aborted November 13, 1930, and since raw milk was being used from the herd he was suspicious that an outbreak was imminent. Several cases of abortion had occurred in the herd in 1926 and 1927 but no cases of undulant fever had developed in the school. The first case of recognized undulant fever appeared on November 26, 1930, about two weeks after the cow had aborted, and the second case (brother of the first) developed while the boys were on Christmas vacation. In the first of these cases the organism was isolated from both blood and feces. Although a number of attempts have been made to isolate organisms from other suspected cases, they have been unsuccessful.

A specimen of blood from each of the cows (about 100) was taken and examined by the physician for agglutinins for the Brucella group. Four were found positive. One was from the cow that had recently aborted. These four cows were disposed of and pasteurization of the milk started. No new cases of abortion have appeared in the herd since.

Shortly after the Christmas vacation, specimens of blood were taken from each of the boys and tests made for presence of agglutinins for the Brucella group. Specimens that were found positive or suspicious were sent to these Laboratories for further examination. About 240 boys in the school and about 60 employees were tested. The 20 cases reported positive by these Laboratories are included in the 27 specimens, 6,686 through 27,572 (Table III).

Specimens 6,686 (27,291 repeat) and 1,685 (organism isolated) were definitely considered by the physician as cases of undulant fever. Specimens 6,854, 6,856, 21,617, and 75,066 he called mild cases since symptoms, although slight, could be considered as indicative of undulant fever. These six cases and one other not reported were the only ones in the group of twenty reported positive

*The authors wish to express their appreciation for the excellent cooperation of this physician. His name is withheld to preserve the identity of the school.

by these Laboratories that were considered by the physician to be indicative of undulant fever.

The 19 specimens, 22,700 through 27,572, were all considered clinically negative. There were no symptoms suggesting undulant fever and, although in some instances upper respiratory symptoms developed, they were usually mild. In six instances there was no illness whatsoever.

The six specimens numbered 2,191 through 76,022 present an interesting case in which, although no symptoms of undulant fever were demonstrable, a definite increase and finally decrease in titer was shown over a period of ten months. The physician has made a number of agglutination tests on this patient. The original examination was routine and only a slight titer was demonstrated. In following the titer in this case over a period of ten months there was demonstrated an increase to a titer of 6000 which finally decreased to 1:120. At no time did the physician note symptoms of undulant fever although the patient did have an upper respiratory infection and mumps during the year.

Following is the case report supplied by the physician:

"Case 26.—Aged eighteen years. During a small epidemic of upper respiratory infection, in May, 1930, this boy was admitted to the infirmary with the first illness which he had had in many years. It was characterized by headache, nasal discharge, and fever (39.6°), but was quite mild as his temperature dropped to normal on his second day, and remained normal until his discharge three days later. Because he happened to be one of a group of persons on whom Brucella agglutination tests were being made in an effort to determine the incidence of such agglutinins among normal persons, his serum was tested at this time. No agglutinins were found. In July, 1930, he had a mild and uncomplicated case of mumps. On January 12, 1931, another routine serum test was made which was positive in 1:2560 dilution. On February 10, 1931, his serum was positive in a 1:5120 dilution. This boy had been perfectly well since his cold in May, except for the mumps. He had no complaint whatever, and no sign or symptom of undulant fever, or of any other illness. A weight chart, recording monthly weighings, had been showing an uninterrupted gain in weight over a two-year period. As soon as the positive agglutination was discovered, temperatures were taken twice daily for two weeks. Then an occasional evening temperature was taken over a period of five months, but, at no time, did he have any elevation above the normal. His stools were repeatedly negative for Brucella organisms, and so was his urine. During the winter, this boy carried a heavy program of studies successfully, and did a great deal of manual labor out-of-doors. He gained six pounds in weight despite the fact that he was somewhat overweight before. His hemoglobin remained at 95 per cent (Sahli). Apparently the only response which this boy made to his Brucella infection was his production of agglutinins. Nearly a year after the presence of agglutinins was first noted, his serum agglutinated Brucella in dilutions up to 1:120."

In some of these specimens, notably 2,494, 6,684, 25,242, 26,914, and 31,392, the agglutinin titers were comparatively low, yet all were well over the arbitrary lower limit of positivity. The possibility that these agglutinations are nonspecific seems to us to be slight, and certainly the reactions on the specimens whose titers run from 750 to 3000 can hardly be considered nonspecific.

DISCUSSION

The value of a diagnostic test in public health laboratories depends not alone upon accuracy in diagnosis but also upon the speed with which the test can be completed. In our former article on a shorter series of specimens, we[1] found the Huddleson "Rapid Method" met these requirements. After further study

over a period of twelve months we are convinced that this method is a distinct improvement over the two macroscopic tube tests with which it was compared.

In a recently published article by Gilbert and Coleman[7] the authors emphasized "the economy of time and glassware," by the use of their presumptive tube test. They further note that the Huddleson rapid method takes "almost as much time as the routine macroscopic tube method." These authors further report that, "the presumptive tests are placed in the water-bath at 55° C. for four hours and then left in the refrigerator overnight before being read." Since a Huddleson test requires as a maximum less than five minutes to complete, we question the "economy of time." Further we cannot conceive any economy of glassware since each test requires one pipette only and no test tubes.

In this investigation we again find greater numbers of questionable reactions with the tube tests. Although there were apparently six more positive reactions in the Conn. tube test than in the Huddleson test, two of these were tests repeated after a lapse of months and although the tube test still remained positive (1:300), the Huddleson had diminished in titer from 1:3000 to 1:100 where it fell in the "questionable" group under our arbitrary classification. Further, the cases upon which the other four tests were made were not considered undulant fever by the physician. Considering our results from a purely laboratory standpoint we feel that the Huddleson "Rapid Method" will increase the efficiency of our undulant fever diagnostic work allowing us to furnish the physician with an accurate report in a shorter time at no greater expense. On the basis of our results the macroscopic tube test has been discontinued in these Laboratories and the Huddleson "Rapid Method" established as a routine procedure.

By the establishment of an arbitrary standard for reporting specimens as positive, we felt that a dilution of 1:300 showing 50 per cent agglutination was sufficiently high to obviate nonspecific agglutinations. When agglutinins are obtained in such a titer a report of "positive for undulant fever" seems conservative.

In our series of specimens for the year, particularly in the group of specimens 6,686-27,572, we come to an impasse: Either we must consider the 1:300 standard as considerably too low or admit the existence of cases of Brucella infection that fail to show recognizable clinical symptoms of undulant fever. It is difficult to conceive with our present knowledge of immunologic phenomena that nonspecific agglutinins in titers as high as the 1:6000 dilution of serum can be present in the blood stream of an individual either ill with "upper respiratory infections" or, more particularly, when no symptoms of any disease exist. Further, it has been reported by Gilbert and Coleman (loc. cit.) that only 0.4 per cent of 81,848 sera received for complement fixation test for syphilis showed agglutinins for Brucella organisms in the 1:80 dilution or higher. These results corroborate those of McAlpine and Mickle[8, 9] who found only 0.6 per cent in a study of 20,000 sera of the same type. Such evidence seems to us to substantiate the value of the positive agglutination reaction as diagnostic of Brucella infection.

In the school where the majority of high-titered agglutinins were demonstrated in the absence of clinical symptoms of undulant fever we know that these individuals had been drinking quantities of raw milk from a herd in which abor-

tion had occurred and, further, from which Brucella organisms were isolated. In itself this seems sufficient evidence to warrant our findings of Brucella agglutinins.

It is true that attempts to cultivate organisms from many of these individuals failed. Even though the demonstration of Brucella organisms in the feces or blood stream is an accepted criterion for diagnosis of undulant fever regardless of clinical symptoms, we do not feel that inability to demonstrate the organism precludes the possibility of such a diagnosis. Those who have made attempts to isolate the Brucella from man realize the extreme difficulty encountered in most instances. From a study of the literature we find dilutions of 1:80, 1:100, and 1:160 accepted as titers on which to base diagnosis, and so we feel that our 1:300 arbitrary standard is conservative.

The case on which a number of tests were made seems to us to be a definite illustration of a Brucella infection in which the symptoms were too obscure and mild to be recognized as such. The case record is reported. Here is an individual who developed agglutinins from a dilution of 1:20 up to 1:6000 and finally decreased to a titer of 1:120. It does not seem probable that a Brucella infection did not exist in this case. The patient did have mumps and a slight upper respiratory infection during the year. However, none of the classical symptoms of undulant fever presented themselves at any time during the rise and fall of agglutinins in the blood stream.

The obvious conclusion is that extremely mild cases with atypical symptoms of undulant fever do exist. This may be due to a low virulence of the organism or possibly can be related to the age groupings. Although our records are not sufficiently complete to warrant a definite statement, there is evidence that undulant fever in children and young adults is milder than in the older groups. In conclusion, it seems probable that undulant fever may not necessarily manifest itself with the classical symptoms but may be associated, either directly or indirectly, with upper respiratory infections, coryza, or, as Borman (loc. cit.) notes, middle-ear infections.

SUMMARY AND CONCLUSIONS

1. The Huddleson "Rapid Method" is an accurate and efficient means for the diagnosis of Brucella infection in man.

2. Fewer questionable reactions are obtained with the Huddleson Rapid Method than with the two tube test methods used.

3. A series of cases are presented in which, although no symptoms of undulant fever were demonstrated, high-titered agglutinins were present.

4. Brucella infections may be present but not recognizable because of mild symptoms, particularly in young adults and children.

5. This investigation furnishes additional evidence for the substitution of the term "Brucella infection" in place of undulant fever.

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A SIMPLE METHOD OF CHECKING VARIOUS CONCENTRATIONS OF SOLUTIONS OF NOVOCAINE HYDROCHLORIDE*

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NEW YORK, N. Y.

IN VIEW of the not infrequent occurrence of cocaine and procaine poisoning from the use of solutions of higher concentrations than intended, it seems important that a practical test be devised for checking the strengths of solutions of these two anesthetics before use.

Such a test in order to be practicable in the pharmacy, operating room or dispensary must be so simple in equipment and technic that it can be carried out by any one without any special training in chemistry, in the minimum of time, and with the minimum of effort. Finally, the end point should be sharp and unmistakable.

The following test, in our opinion, fulfills the above qualifications.

The Test.—The test consists of taking 2 c.c. of one reagent, adding 5 drops of the indicator, shaking, then adding 1 c.c. of the anesthetic solution to be checked, and shaking again. The resulting color change is compared with the standard chart. The average time for performing this test is thirty-five seconds.

The Reagent.—A N/10 solution of sodium carbonate solution is made according to this formula:

Anhydrous sodium carbonate (C. P.)	5.3 grams
Distilled water	1000. c.c.

This solution must be made with fair accuracy. Once made, it can be used for a long time.

The Indicator.—In order to cover the wide PH range involved in tests for the

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*From the Laboratory of Surgical Research, New York University and Bellevue Hospital Medical College, New York.
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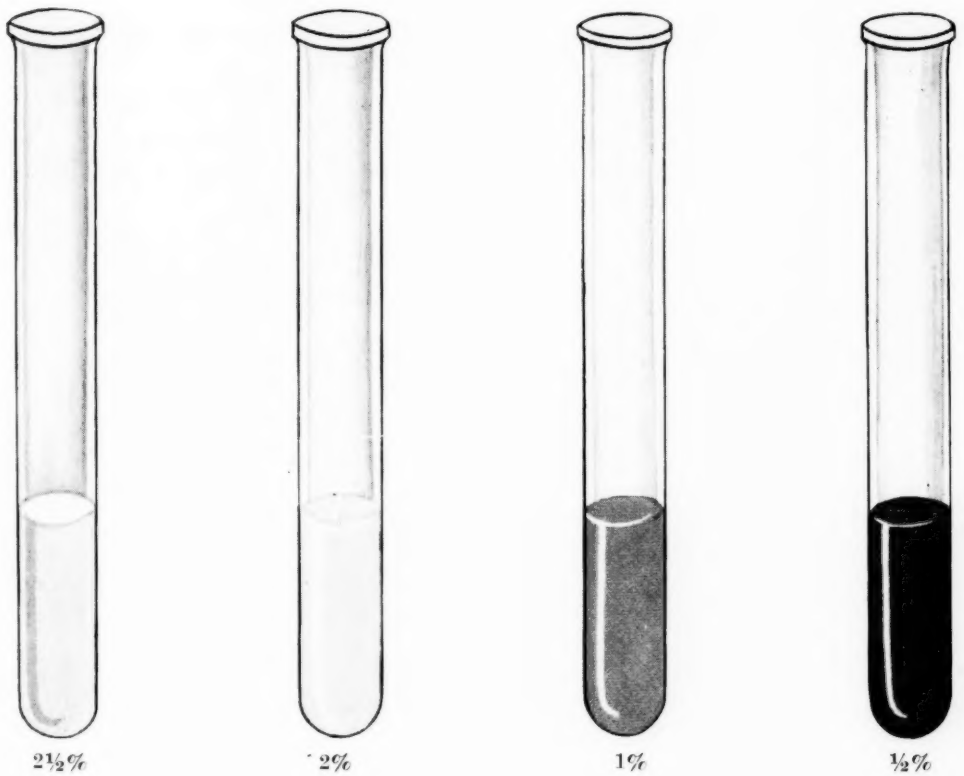

Fig. 1.—Color chart for tests with different concentrations of novocaine hydrochloride.

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common strengths of procaine hydrochloride used in surgical practice, a mixture of two indicators was found necessary. It is made according to this formula:

Thymophthalein	1.0	gm.
Phenolphthalein	0.050	gm.
Alcohol (70%) to make	100.	c.c.

Apparatus.—

(1) Test tubes.

(2) A 2 c.c. syringe for measuring out the reagent.

(3) A 1 c.c. tuberculin syringe for measuring out 1 c.c. of the solution to be tested.

(4) A chart of standard colors, which may be made by a local artist.

The Technic.—Was sufficiently described above.

The Color Changes.—The color developed in the tube by the addition of 5 drops of the indicator is deep blue. The color changes resulting from the addition of 1 c.c. of various concentrations of procaine hydrochloride are shown in Fig. 1. Comparison should be made in reflected artificial light.

It will be seen that any concentration higher than 2 per cent renders the resulting mixture colorless. Intermediate strengths cause the development of intermediate shades. A definite color is produced with one drop of the mixed indicator, but because of the fact that the color developed in transmitted light is different from that in reflected light, 5 drops of the indicator are used to intensify the color to such an extent that light transmission through the solution is largely eliminated.

By varying the amount of reagent used or of the solution to be tested, the strengths of solutions above or below these covered by the color range of the chart can be approximately determined. Thus, if a 1 c.c. solution produces only a slight change, doubt might be aroused as to whether it is a ½ per cent solution or whether it contains any procaine at all. By reducing the quantity of the reagent to 1 c.c., 1 c.c. of a ½ per cent solution should bring on a color change similar to a 1 c.c. of a 1 per cent solution made with 2 c.c. of the reagent. Again, a solution, 1 c.c. of which entirely decolorizes the testing mixture, is of a percentage above percentages given in the color chart given, and one is left in doubt as to the actual strength of the solution. If, by reducing the amount of the solution added from the standard 1 c.c. to ½ c.c. or ¼ c.c., a color change is brought about that matches that produced by 1 c.c. of a 2½ per cent solution in the standard test, then the solution tested is approximately 4 per cent or 8 per cent. The same result can be accomplished by keeping the solution to be tested at 1 c.c., but by doubling or quadrupling the reagent.

Cocaine hydrochloride, because it is somewhat more acid than procaine hydrochloride, produces color changes slightly more marked than, although still comparable with, those produced by procaine hydrochloride.

We wish to thank Dr. Isidor Greenwald, of the Department of Biochemistry, and Dr. Kenneth C. Blanchard, of the Department of Biology, for their valuable suggestions. Grateful acknowledgment is hereby made of the kind encouragement of Professor George David Stewart and of Dr. Arthur M. Wright, of the Department of Surgery.

A LABORATORY INHALATION TECHNIC FOR THE COMPARISON OF SEMIVOLATILE LIQUIDS

DAVID A. BRYCE, M.D., PLAINFIELD, N. J.

ATTEMPTS have been made by various independent investigators to develop a standard technic for the absolute and comparative biologic assay of liquids boiling above the body temperature and below 100° C. Fieldner, Katz, and Kinney[1] published an exhaustive study of such liquids, to which I have referred for complete toxicity figures and other data. A similar report was made in conjunction with the Carbide and Carbon Chemical Corporation by the Bureau of Mines, reported through Sayers, Yant, Waite, and Patty,[2] in which they used rather elaborate gas chamber apparatus and gauze wicks, blown by an electric fan, for vaporization. The Chemical Warfare Service has extensive information upon this subject. Photostats of three types of their apparatus have been studied by me, for which I desire to acknowledge the great courtesy of Colonel C. E. Brigham, Commanding Officer of Edgewood Arsenal, and Major Linthicum of the same post.

In brief it has seemed to me that the various methods previously described have partaken of at least one of the following disadvantages:

(a) too elaborate and/or costly apparatus.

(b) inaccurate methods of vaporization.

(c) failure to consider adequately the CO_2 content of the gas in the lethal chamber.

It has been impossible for me to review the entire world literature upon this subject. The technic herein described is believed to be new; and is certainly original, as it was designed by the writer to solve a laboratory technical problem before any literature on the subject was read. For aid in this work we are indebted to Dr. A. A. Tichnor, Mr. George Hedden, Mr. Lincoln Shafer; and to Mr. Bertus Schnucker for great assistance in conducting the first portion of the work.

The apparatus was designed to provide "open-drop" conditions. That is to say, in the lethal chamber there was to be complete and immediate evacuation of carbon dioxide and of the exhaled gases as well as a free sweep for the unexpired gases. This end was obtained by operating the apparatus at a rate of about 1500 c.c. per minute. The factor of adsorption of the various agents to the sides of the lethal chamber or to the skin of the mouse was controlled by frequent cleaning of the chamber with dry tissue, and by using only one mouse at a time in the lethal chamber except in a few of the ether experiments. The apparatus was designed with a view to placing at the disposal of any ordinary chemical or biologic laboratory, by means of routine chemical equipment, a method for the comparative biologic assay of semivolatile liquids.

The apparatus, a diagrammatic sketch of which is attached herewith, may be explained as follows: Flask A is the point of entrance of air at room temperature. In this Erlenmeyer suction flask the bottom is covered to a depth of about two inches with clear, medical, filtered, liquid petrolatum. The intake tube fits just be-

low the surface of this so that air entering the chamber is filtered through the petrolatum and yet does not take up any additional moisture. The air then enters a calcium chloride tube, *B*. From here it passes along the line to a copper coil, *C*, which is immersed in an oil bath. This oil bath is on an electric heater. The air passes from the copper coil along a new line and through a second calcium chloride tube. The air then enters a bead column lying within a steam jacket at a point parallel to the point at which a burette enters the bead column. The burette, *D*, contains the agent with which we worked, and is connected by a tube, *P*, to a compressed air inlet, so as to maintain a positive head of pressure on the liquids at all

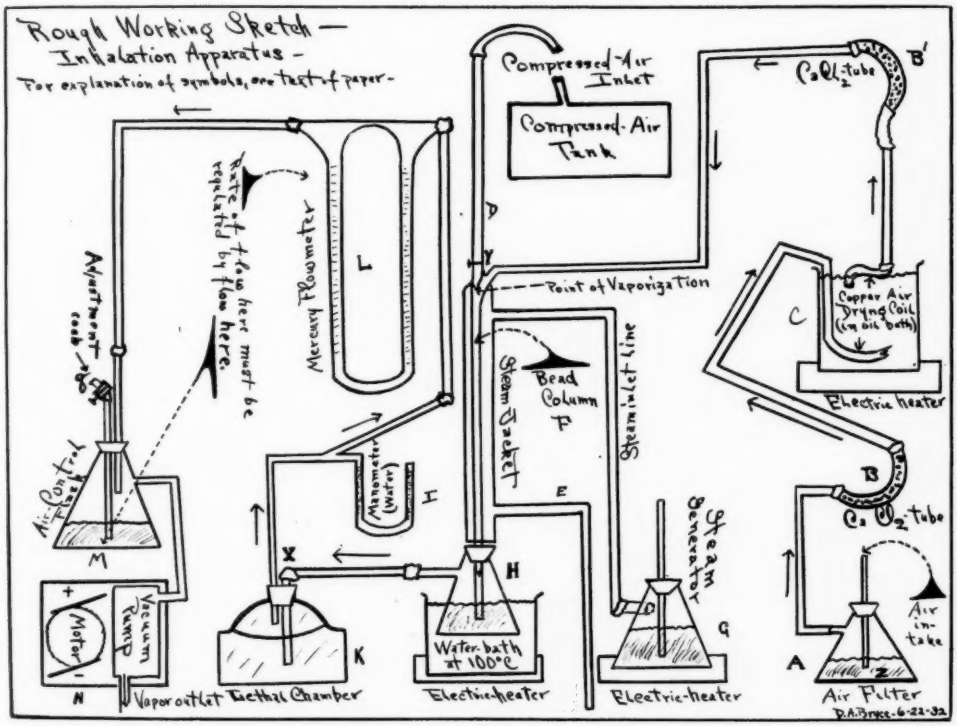

Fig. 1.

times. The air, and the liquid in minute amounts, pass over the bead column which is heated to any desired temperature up to 100° C. by the steam jacket, passing slowly down to vaporize the liquid completely, so that when it enters the Erlenmeyer suction flask, *H*, there should be absolutely no condensation in the tube and none upon the bottom of the flask. The flask is immersed in a water-bath which in turn is heated by an electric heater. Air passes from the Erlenmeyer flask by the suction nozzle and is transmitted directly to the lethal chamber. The latter, *K*, is a small desiccator jar with a perforated rubber cork. The air passes out from the lethal chamber, *X*, to a line into which is placed a water manometer. This manometer, *I*, merely assures proper conditions in the chamber for the mouse. At this point the air proceeds through a flowmeter, *L*, which gauges the evenness and flow of the high vacuum pump. The air goes from this flowmeter to an Erlenmeyer flask which is connected by its suction nozzle, *M*, directly to the high vacuum pump,

N. This flask, *M*, serves as a very useful means of regulating the air flow by adjustment of the tube, *U*.

It will be noted that by this arrangement standard animals may be weighed and quickly transferred to the experimental chamber under absolutely standard conditions which are constant and controlled; at pressures which are absolutely constant; and under conditions which assure the animal immediate evacuation from the lethal chamber of expired carbon dioxide, and its replacement by air containing its full quota of oxygen. It was found that normal standard mice placed in the lethal chamber with a flow of 1500 c.c. pure air per minute were perfectly comfortable for an indefinite period. Several mice were tried out in this way for periods of forty minutes, and no change was observed.

Certain precautions must be observed in the operation of this apparatus. It will be noted that we have employed an open flame at no point; of course, the reason for this is obvious. At point *Z*, namely the liquid in the air control flask, one must constantly observe the evenness of the flow. This furnishes an index as to whether there is any leakage in the system. If leakage is occurring of course the flow will diminish or cease in the flask. It is not practicable to insert a flowmeter at this point, since if one does so the force necessary to flow through the flowmeter detracts from the final pressure, and changes the reading for the system. At point *Y* the stopcock on the burette must be constantly watched. The flow must be adjusted very finely, for work of this kind. In our work we used a flow of 0.15 c.c. per minute for most of the work. Point *X* had best be enclosed in a rubber tube of some sort, preferably vulcanized or sealed at the top. Any leakage at *K* or *X* will, of course, invalidate the work, and is promptly apparent in the abnormal action of the air control flask. If these precautions are properly attended to, the apparatus forms a convenient and easy method of laboratory procedure.

A note concerning the suitability of white mice for this sort of work may not be ill-timed. In my somewhat brief experience it has appeared that vapors which act entirely or essentially as central nervous toxic agents are well-adapted to use with white mice for toxicity determinations. On the other hand, such liquids as seem to directly affect the heart and kill through this organ, such as chloroform, appear to be unsuited for use with white mice in inhalation apparatus. This is a purely preliminary conclusion, if it may even be called such.

The sole excuse of my publishing these notes has been the hope that it may aid other laboratory workers who are faced with some practical problem and find it impossible for economic or other reasons to manufacture an elaborate apparatus involving gas chambers, etc.

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NOTE ON THE SUMNER METHOD FOR SUGAR IN URINE*

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SUMNER[1] in 1925 reported a revised method for the determination of sugar in urine in which he employed the following reagent:

To 10 gm. of crystallized phenol add 22 c.c. of 10 per cent sodium hydroxide. Dissolve in a little water and dilute to a volume of 100 c.c. Weigh out 6.9 gm. of sodium bisulphite and add to this 69 c.c. of alkaline phenol solution. Now add a solution containing 300 c.c. of 4.5 per cent sodium hydroxide, 255 gm. of Rochelle salt ($KNaC_4H_4O_6 \cdot 4H_2O$) and 880 c.c. of 1 per cent dinitrosalicylic acid solution. Mix and keep tightly stoppered in well filled bottles.

The method was described as follows:

Pipette into a Folin-Wu sugar tube 1 c.c. of urine (diluted if necessary) and 3 c.c. of the reagent. Mix and heat 5 minutes in boiling water. Cool three minutes in running water, dilute to 25 c.c. volume, mix, and compare in colorimeter with standard prepared with 1, 0.5, or 0.25 mg. of glucose, according to the concentration of sugar in the urine.

This procedure has been employed by us in the routine analysis of several hundred thousand urines over the past four years. Results have been satisfactory and generally consistent when compared to other standard quantitative methods. For instance the analysis of 100 urines containing sugar in which a comparison was made with Benedict's copper method gave the following averages: Sumner's 2.80 per cent, Benedict's 2.79 per cent. Good agreement was also found in individual analyses. It was found however that with an occasional glycosuric specimen, the reagent gave a result far too low or failed to detect sugar altogether. In one particular case a highly acid urine containing 0.6 per cent sugar gave a result of less than 0.13 per cent. The addition of more alkali to the reagent increased the result to the actual amount. This was reported to Dr. Sumner who, in a private communication suggested the use of 50 per cent more alkali. He also suggested that this might increase the values for normal amounts of sugar in urine. Some experimental results with increased alkali are reported in Table I.

TABLE I

RESULTS WITH INCREASED ALKALI ON GLUCOSE STANDARDS

GLUCOSE STANDARD	COLORIMETER READINGS	
	INCREASED ALKALI	ORIGINAL REAGENT
Per cent	mm.	mm.
0.13	20	22.9
0.20	10	13.0
0.30	10	12.2
0.40	10	10.8
0.50	10	10.9

It will be observed from Table I that increase of alkali definitely increases color production in pure aqueous solutions of glucose.

*From the Laboratories of the Life Extension Institute.
Received for publication, May 31, 1932.

Analysis of urine specimens containing varying amounts of sugar were made, to compare the results of the original reagent with the one to which additional alkali had been added. The figures are given in Table II. It will be noted that, although in occasional instances there was good agreement, in the majority of instances the results with increased alkali were higher.

TABLE II

URINE SPECIMEN	ORIGINAL REAGENT	REAGENT WITH INCREASED ALKALI	URINE SPECIMEN	ORIGINAL REAGENT	REAGENT WITH INCREASED ALKALI
	per cent	per cent		per cent	per cent
1	0.031	0.046	31	0.140	0.240
2	0.065	0.080	32	0.150	0.190
3	0.067	0.094	33	0.270	0.290
4	0.030	0.048	34	0.240	0.260
5	0.094	0.140	35	0.370	0.370
6	0.065	0.072	36	0.130	0.170
7	0.023	0.027	37	0.250	0.320
8	0.079	0.080	38	0.150	0.170
9	0.098	0.100	39	0.130	0.190
10	0.190	0.210	40	0.140	0.170
11	0.160	0.180	41	0.250	0.280
12	0.180	0.210	42	0.240	0.270
13	0.170	0.170	43	0.120	0.180
14	0.140	0.210	44	0.140	0.190
15	0.150	0.260	45	0.150	0.250
16	0.160	0.230	46	0.150	0.150
17	0.130	0.210	47	0.210	0.260
18	0.120	0.190	48	0.270	0.300
19	0.180	0.230	49	0.210	0.260
20	0.170	0.230	50	0.170	0.210
21	0.250	0.300	51	0.160	0.180
22	0.160	0.180	52	0.140	0.220
23	0.190	0.200	53	0.160	0.250
24	0.300	0.350	54	0.160	0.200
25	0.180	0.250	55	0.120	0.140
26	0.150	0.270	56	0.150	0.180
27	0.280	0.320	57	0.110	0.180
28	0.170	0.190	58	0.120	0.180
29	0.120	0.180	59	0.130	0.150
30	0.140	0.180	60	0.150	0.160

A further comparison was made in which Benedict's quantitative copper method was included. The figures which are shown in Table III, indicate that in undiluted urine the reagent with increased alkali gives results which compare favorably with Benedict's method when the values are between 0.6 and 1.33 per cent; also that the results with the original reagent are generally too low. When urine is diluted even to as small a degree as an equal part with water, the original reagent gives fairly good agreement. Presumably this is because dilution diminishes the concentration of acid. This explains why such good average agreement was obtained between the original Sumner's reagent and Benedict's copper method in our first comparisons on 100 glycosuric specimens as noted above and the need for increased alkali was not then detected. Most of these urines contained sufficient sugar to make dilution a necessity.

TABLE III

UNDILUTED URINE	BENEDICT'S	SUMNER'S STANDARD	SUMNER'S INCREASED ALKALI
1	0.6	0.52	0.58
2	0.6	0.53	0.55
3	1.2	0.71	1.2
4	0.66	0.49	0.63
5	0.88	0.62	0.9
6	1.0	0.79	1.08
7	0.7	0.59	0.65
8	1.0	0.59	0.98
9	1.01	0.66	0.98
10	1.33	0.98	1.23
11	0.7	0.56	0.7
12	1.0	0.57	0.99
DILUTED URINE*			
1	0.7	0.78	0.93
2	0.8	0.82	1.05
3	0.64	0.6	0.71
4	1.1	0.96	1.2
5	1.0	0.9	1.01
6	1.05	0.92	1.15

*Equal parts with water.

Although some results with the increased alkali exceed those with Benedict's reagent and may be somewhat too high, we feel that Sumner's method as modified is in the main reliable and an excellent procedure for routine clinical work. It is our custom to match all tubes with standards in similar tubes and consider those showing reductions of less than 0.13 per cent as having negligible amounts of sugar. Those having amounts between 0.13 and 0.5 per cent are read off and reported directly from the test tube standards. Those above 0.5 per cent are determined by the use of Benedict's copper method.

The writer is indebted to Miss Celia Posen, B.S., and Mr. Arthur Gallagher, B.S., for most of the technical work reported in this article.

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ADAPTATION OF THE LEITZ ULTROPAK FOR RAPID TISSUE DIAGNOSIS*

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COMPARATIVELY few clinics have perfected frozen section technic to the extent that a fairly accurate pathologic diagnosis is available for the surgeon in five minutes or less; the practical advantage to the surgeon of such a procedure is obvious. The method of Terry seemed to be a step forward in the rapidity with which tissue might be prepared for examination in the clinic not specially trained for frozen section work; using a special polychrome methylene blue with razor-sectioned fresh tissue, the surface cells were stained and were visible under the microscope by reflected light. In Terry's hands this method proved very satisfactory and fully as accurate a diagnosis could be given in a shorter time than with frozen section; the pathologist not accustomed to interpretation of the type of histologic picture obtained through this medium, however, often felt hesitant to give a definite opinion; illumination difficulties presented an obstacle; the polychrome stain was not clear-cut with tissues containing a considerable amount of fat, for example, breast.

We regarded the Ultropak apparatus, recently developed by Leitz, as a possible solution to rapid tissue diagnosis; our experiments with it in this direction proved successful.

The Ultropak illuminator (Fig. 2), when attached to the microscope stand in place of the usual nose piece (Fig. 1), provides illumination of the surface of opaque objects by incident pencils of rays lying entirely outside the path of the rays of the microscope. It is possible to illuminate and examine not only a surface but to view successive strata and deep structures regardless of overlying strata. A wide variety of objectives (dry, water and oil immersion) are available; objectives can be changed rapidly without readjustment of illuminant.

TECHNIC

1. *Sectioning.*—The fresh tissue is cut to a thickness of 1 to 3 mm. with a *sharp* knife (a light microtome knife or straight razor set aside for this purpose is most satisfactory). The knife is drawn at an angle of 15 degrees from the horizontal rapidly through the tissue, with the distal end of the knife resting on the cork board; care must be exerted to avoid a down-thrust, that would crush the tissue. An effort to obtain a uniformly flat surface is well repaid by the subsequent ease in examination. Extremely soft tissue may be dropped into hot formalin for thirty seconds to one minute to permit greater ease in cutting.

2. *Staining.*—The section is placed in a Gooch crucible (or similar container with a perforated bottom) and dipped into a staining dish containing 0.3 per cent

*From the Division of Pathology of the Philadelphia General Hospital and the Department of Pathology of the University of Pennsylvania.
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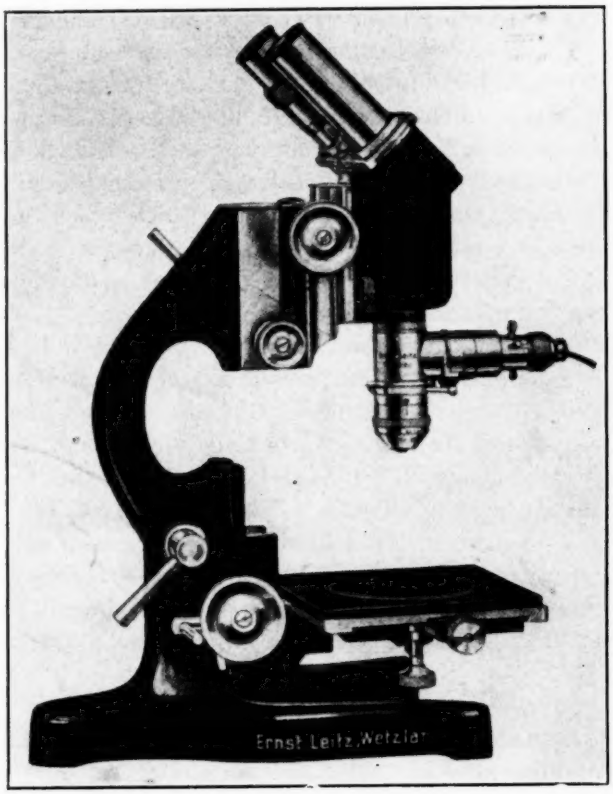

Fig. 1.

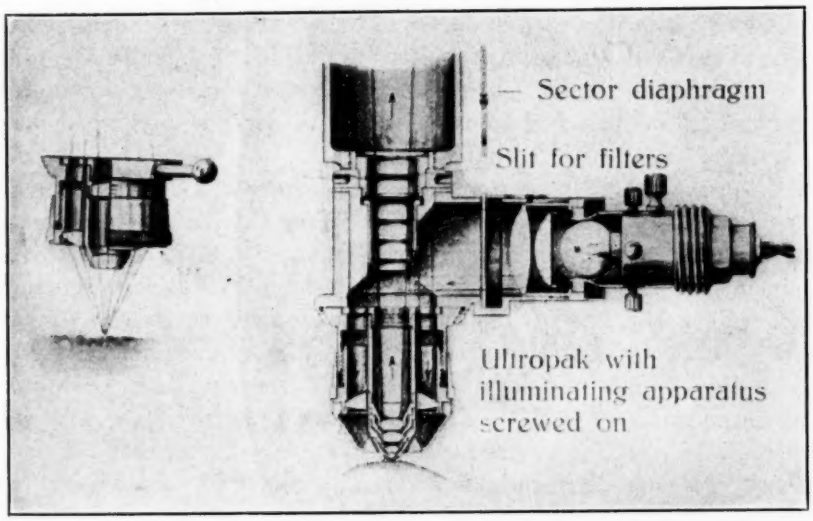

Fig. 2.

toluidin blue; the section should be moved continuously in the stain and brought into view occasionally that the intensity of the stain can be observed. When the surface is stained a uniform blue (of a degree that must be determined by practice,

requiring usually about twenty to thirty seconds), the crucible is withdrawn and dipped into slightly acidulated distilled water (one drop of concentrated hydrochloric acid to 100 c.c. water) until the stain ceases to come away in a cloud. The tissue is transferred to a watch-crystal and immersed in distilled water so that the surface is barely covered and the tissue does not float. (This is accomplished by adding water from a pipette until the fluid level approximates the level of the upper surface of the tissue, then placing a final drop directly on the sectioned surface.) The tissue is now ready for examination; the crystal is placed on a white card on the stage. To insure stability of the watch-crystal during examination, it should be cemented to the concavity of a hanging-drop slide.

3. *Examination.*—We have found the 6.5× dry objective best for a general outlook over the tissue (the 5× with immersion attachment is also useful in some cases). If reflections prove troublesome, a cover slip may be laid across the tissue for low-power examination, being careful to avoid air-bubbles. Sufficiently high magnification for cytologic detail is obtained by removing the cover slip and applying the 23 water-immersion objective. Focus is reached first with the coarse adjustment, sharpness of image being brought out by circular rotation of the objective. The intensity of light must be adjusted with the rheostat control; too low or too great an illumination blurs the field. The Bausch and Lomb (No. 1154) wide-field ocular is an almost essential adjunct.

DISCUSSION

The average time required for preparation of tissue for examination by this method is two minutes. The histologic picture is interpreted as readily and accurately as that given by frozen section; a far greater area is available for inspection than with the latter method. Using toluidin blue, nuclei are clear-cut and cytoplasm well outlined; a certain degree of polychrome effect is obtained, in that the stroma takes a pale pink stain as opposed to the blue of the cells. Well-ripened polychrome methylene blue of Unna gave good results but was not as uniform as the toluidin blue; we have had no experience with Terry's polychrome stain, but believe it useful except with fatty tissue.

The following tumor tissues were diagnosed correctly by Ultropak examination (confirmation of the preliminary diagnoses being obtained by examination of paraffin sections): *Skin:* squamous-cell carcinoma (Types I, II and III), basal-cell carcinoma. *Lip:* squamous-cell carcinoma (Type II). *Tonsil:* squamous-cell carcinoma (Type II). *Lung:* metastatic squamous-cell carcinoma (Type III). *Stomach:* adenocarcinoma (medullary). *Sigmoid:* adenocarcinoma (Type II). *Rectum:* benign villous papilloma. *Prostate:* adenocarcinoma. *Cervix:* squamous-cell carcinoma (Type IV). *Breast:* adenocarcinoma (Type IV), spindle-cell sarcoma. *Eye:* melanoma sine melanin. The single tumor that did not permit positive identification, although its malignant character was obvious, proved subsequently to be lymph node metastasis from an adenocarcinoma of the stomach. Several ulcerative lesions of the skin, diagnosed clinically as malignant, were observed to be inflammatory in character; one of these latter was suspected by Ultropak examinations to be gumma; this was proved later to be true. Unfortunately, so-called "border-line cases" were not encountered during the short period

of time allotted to these observations. Fresh autopsy material (i. e. heart, liver, kidney, etc.) was examined and a rather clear picture obtained; it was found that, in the latter case, fixation in hot formalin gave a better staining reaction and was to be preferred, the time element not being important.

It is hardly necessary to add that increased surety of diagnosis comes through repeated correlation of the histologic picture obtained by the Ultropak and by paraffin section.

I am indebted to Mr. J. P. Vollrath, of Street, Linder and Propert, for the use of the Ultropak apparatus for this work.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

SERUM PROTEINS, In Infancy and Childhood, Webb, C. H. Am. J. Dis. Child. 44: 1239, 1932.

1. Older children have normal values for serum protein at the same level as those in the adult. Infants have low values for total protein and particularly low values for serum globulin; this possibly has some relation to hydrolability or to absence of the infectious diseases.

2. No regular relationship between the state of nutrition and serum albumin content could be established.

3. Acute infections produce comparatively mild and temporary changes in the serum proteins.

4. Alterations in the concentration of serum protein during chronic infections depend on the type of infection. In the series in question the most pronounced changes were seen in (a) chronic osteomyelitis, which was marked by elevated values for globulin, and (b) renal involvement, especially nephrosis, characterized by lowered serum albumin and lowered total proteins but increase in globulins.

DIPHTHERIA, Immunization Against With Toxoid, Kreitz, P. B. Am. J. Dis. Child. 44: 1249, 1932.

Nurses and hospital employees received two 1 c.c. doses of commercial toxoid with seven days between injections. Immunity, measured by a negative Schick test, was present in from 80 to 87 per cent after from fourteen to sixteen weeks.

Immunity, measured by a negative Schick test, was acquired rapidly. One week after the second injection of toxoid, with an interval of one week between injections, from 34 to 43 per cent of the subjects gave negative reactions; at two weeks, from 41 to 46 per cent; at three weeks, from 61 to 63 per cent; at four weeks, from 71 to 73 per cent, and at from fourteen to sixteen weeks, from 80 to 87 per cent.

A single injection of 1.5 c.c. of commercial toxoid did not give sufficient immunity to warrant its continued use in comparison with the immunity obtained by two injections of 1 c.c. of toxoid at an interval of one week.

Data are here presented that tend to show that it is probable that a total dosage of at least 2 c.c. of commercial toxoid is necessary for satisfactory immunization against diphtheria.

CYANOSIS, In the New-Born, Hunt, F. C. Am. J. Dis. Child. 44: 1268, 1932.

By analysis of the results obtained at one hundred and eighteen postmortem examinations, an attempt has been made to ascertain the more common pathologic lesions causing cyanosis in new-born infants. It is realized that the causes of cyanosis and of death are not synonymous, and that several factors may be involved. Hemorrhage, whether intracranial, pulmonary or intra-abdominal cannot be disregarded as a contributing cause in many instances.

Intracranial lesions, although frequently found at postmortem examination, are not always of sufficient severity to produce symptoms. Especially is this true of small tentorial tears, which must often be disregarded. On the other hand, extensive unilateral or bilateral tears of this supporting structure, accompanied as they most frequently are by profuse hemorrhage, would seem sufficient cause for cyanosis. Meningeal bleeding may occur in the absence of tentorial lesions, and is often profusely distributed over the cerebral hemispheres or at the base. These two types of lesion, namely severe tentorial tears and meningeal hemorrhage are

usually the direct result of birth trauma, hence cyanosis will appear within the first few days of life.

The cyanosis accompanying congenital cardiac disease is due to the mixture of venous with arterial blood or to stasis. With such abnormalities, the blueness may vary directly with the activity of the infant and not appear until some weeks after birth. Unless the lesion is severe life is possible, in some cases well into childhood. The relatively few cases that appear in this series are therefore not indicative of the true frequency of cyanosis as a manifestation of congenital cardiac abnormalities.

Pneumonia is the most common single lesion found in these infants. The disease does not differ from that seen in older children, except for the variability of the signs. The appearance of a hyaline membrane, which is probably the result of aspirated amniotic fluid, is more common than was formerly believed. In small amounts, this is probably of little significance. In the cases in which it forms a membrane lining the alveoli of large sections of the lung, it cannot help but cause respiratory embarrassment. Persistent cyanosis appears early in the life of these infants, and may be the only sign present.

Other pathologic conditions may produce cyanosis in the new-born, but they are relatively infrequent. The greatest difficulty in diagnosis is usually between pulmonary and cerebral lesions. Lumbar puncture or roentgenologic examination of the chest may be of assistance in establishing the cause of cyanosis, but not infrequently may give no definite aid. In the infants coming to postmortem examination, intracranial lesions, including both hemorrhage and tentorial tears, are the most frequent cause of cyanosis, but, as a single severe entity, pneumonia is the predominant factor.

BLOOD SEDIMENTATION, Increased Suspension Stability of Erythrocytes, Schulhof, K. J. A. M. A. **100**: 318, 1933.

A very slow sedimentation rate of the erythrocytes is a constant symptom in allergic individuals unless they show evidence of a complication, especially infection, which tends to accelerate the sedimentation. As an infection may mask the expected slow rate, so may an allergic individual show a slow rate even in the presence of a serious infection of short duration, such as a gangrenous appendix. The interaction of the two opposite tendencies should be taken into consideration in the diagnostic and prognostic evaluation of the sedimentation rates. Sudden postoperative mishaps may occur in patients with abnormally slow rates.

Besides the patients with known allergic conditions, a very slow sedimentation rate seems to be fairly constant among patients with lumbago. Liver treatment seemed to account for the slow sedimentation rates in other patients.

The frequency of very slow sedimentation rates among apparently nonallergic close relatives of allergic patients is striking and deserves a closer investigation.

A difference in the technic may partly explain the fact that the slow sedimentation rates have been overlooked clinically.

MILK: Yeast Medium for Determining the Bacterial Content of, by the Plate Method, Devereux, E. D., and Etchells, J. L. Am. J. Public Health **23**: 149, 1933.

This medium differs from plain nutrient agar in that yeast extract and peptonized milk are substituted for meat extract and peptone, and dextrose is added.

Formula of Yeast Extract Agar

Yeast extract (Difco)	5 gm.
Peptonized Milk (Difco)	10 gm.
Salt	5 gm.
Dextrose	10 gm.
Agar	15 gm.
Distilled water	1000 c.c.

Adjusted to P_H 7 and autoclaved at 15 pounds for fifteen minutes.

This medium when used in series with the plain agar described in Standard Methods for Milk Analysis gave counts at the end of twenty-four hours' incubation which were on

the average comparable to plain nutrient agar counts made at the end of forty-eight hours, resulting in a saving of twenty-four hours for the completion of the test. Also it gave counts at the end of forty-eight hours which were on the average 45 per cent higher than similarly made plain agar counts.

MELANURIA, Blackberg, S. N., and Wanger, J. O. J. A. M. A. **100:** 344, 1933.

The following method for the detection of melanin in the urine is described as more reliable than those in common use:

1. A twenty-four hour specimen of urine is evaporated to one-fourth of the original volume.

2. One gram of potassium persulphate is added for each 100 c.c. of the concentrated urine.

3. At the end of two hours, an equal volume of absolute methyl alcohol is added. The precipitated melanin is allowed to settle.

4. The precipitate is filtered off and washed with water until the washings are colorless, then washed with methyl alcohol, to remove any soluble pigments remaining. Finally, it is washed with ether. If the test is positive, there remains on the filter paper a brownish black precipitate, which can be dissolved off with alkali, most conveniently with 5 per cent sodium hydroxide. Acidification of the alkalin solution causes a reprecipitation of the melanin.

PNEUMOCOCCUS, Direct Typing of, Valentine, F. C. Lancet **224:** 22, 1933.

Referring to the method described by Armstrong (and abstracted in the May, 1932, issue of this JOURNAL), Valentine describes a method whereby the "neutralized" capsules may be made easily recognizable.

Three or four loopfuls of the type serum are mixed on a slide with a loopful of sputum and covered with a cover glass the edges of which are sealed with vaseline.

After standing for twenty to thirty minutes the cover glass is slid off and the film on the slide allowed to dry. The bulk of the vaseline is scraped from the slide and the remainder removed by wiping with xylol.

The remaining steps of the procedure are as follows:

1. Wash the film gently with tap water (to remove serum).

2. Stain for two to three minutes with dilute carbol-fuchsin (1 part of filtered stain to 5 or 6 parts of water).

3. Wash in tap water and counter-stain ten seconds with carbol-thionin (sat. sol. of thionin in 50 per cent alcohol 1 part; 5 per cent phenol solution 9 parts. Safranin may be used instead of carbol-thionin but the staining is less intense).

4. Wash in tap water, blot dry, and examine.

Bacteria stain practically black and all else red. The capsules of pneumococci treated with homologous serum appear quite large and stain a strong red; "unneutralized" capsules do not stain.

REVIEWS

Books and Monographs for Review should be sent direct to the Editor, Dr. Warren T. Vaughan, Professional Building, Richmond, Va.

Bright's Disease*

FOR several years we have in a measure been marking time in our understanding of the pathology and progress of Bright's Disease. This has been due to the fact that by clinical studies we were unable to prognosticate the type of renal lesion that would be found at autopsy. As a consequence the histopathology was to a certain extent neglected by clinicians and attention was directed more to clinical functional studies during life. The work of Addis and the authors of the present monograph has however shown that careful clinical studies during life will enable the physician to visualize the nature of the renal lesion.

The authors accept Addis' clinical classification into hemorrhagic Bright's Disease, arteriosclerotic Bright's Disease and degenerative Bright's Disease. They find that these correspond in general to the pathologic lesions of glomerular inflammation, arteriolar disease and degeneration of the tubular epithelium. They reconcile Addis' clinical observations with the pathological studies of Volhard and Fahr.

The remainder of the volume is devoted to clinical and pathological studies with antemortem functional observations and postmortem structural observations on a series of individuals with Bright's Disease. The authors conclude that the three types of Bright's Disease are essentially different in their genesis and pathological nature. They find, contrary to the general belief, that gradual decrease of urea excreting ability frequently develops during the course of nephrosis. The disease may even end in uremia. In this case the glomeruli are involved in the terminal changes. They conclude, with Addis, that observations during the course of the disease will enable one to deduce the general nature of the pathological changes. Of the different features of the disease that were followed, the blood urea clearance proved to be the most closely related to the onset of final renal failure. The clearance could remain indefinitely at 10 per cent of normal, without uremia, but when it fell below 5 per cent uremia occurred.

This volume together with that by Addis and Oliver, represent the latest and most authoritative expositions on the pathology of Bright's Disease.

Cancer†

DR. WILLY MEYER, himself a surgeon of eminence, devoted the best efforts of his productive life to a study of the problem of cancer. He recognized that while surgery and x-ray and radium are the most helpful means available for combating this affliction, none of them have solved the problem of the control of cancer. This monograph sums up the present situation, reviews the recent advances particularly in the study of the general metabolism and physiology in cancerous individuals, and presents in detail the author's theory of the origin of the disease. He goes further and outlines the program of treatment based upon his theory.

Whether or not the future will show that his theory is correct, his monograph represents a milestone.

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*Bright's Disease. Observations on the Courses of Different Types and on the Resultant Changes in Renal Anatomy. By D. D. Van Slyke et al. From the Hospital of the Rockefeller Institute for Medical Research. Medicine Monographs, Volume XVIII. 66 Charts, four color plates, 36 halftone figures, cloth, pages 130. Williams and Wilkins Company, Baltimore, 1930.

†Cancer. Its Origin, Its Development and Its Self-Perpetuation. The Therapy of Operable and Inoperable Cancer in the Light of a Systemic Conception of Malignancy. A research by Willy Meyer, M.D., Consulting Surgeon to the Lenox Hill and Postgraduate Hospitals, New York Infirmary for Women and Children, etc.; Emeritus Professor of Surgery, N. Y. Postgraduate Medical School. Cloth, pages 427. Paul B. Hoeber, Inc., New York, 1931.

The author feels that we will eventually reach a stage at which we can determine the presence of cancerous susceptibility and can inaugurate appropriate treatment prior to the onset of the actual cancer. He feels that cancer is a systemic disease, the resultant of two simultaneously active chronic irritations, one systemic and the other local. Neither alone will produce cancer but both acting in conjunction are responsible for the disease. The systemic chronic irritation has its origin in the irregularities of the nervous system and disturbances of the physicochemical condition of the body fluids and the local irritation has its origin in an abnormal behavior of the tissue cells. The systemic lesion disturbs the sympathetic-parasympathetic balance thereby upsetting the parathyroid-pancreas balance which in turn alters the normal potassium-calcium ratio of the serum, in favor of potassium. Calcium deficit in turn alters the sympathetic nervous function thereby closing a vicious circle. The result is an increasing serum alkalosis. This produces a hydrophobic condition of the cells, which impairs healing capacity. A person in this condition is cancer susceptible.

The local predisposition also produces a local hydrops and alkalosis which tend to prevent healing. The local necrobiotic products stimulate cell reproduction.

He describes four phases in the progress of the disease, the first the phase of cancer susceptibility in which the systemic factors are at work and there is a blood alkalosis, an innocent condition which should be remediable; second the phase of presence of local chronic irritation, also innocent and remediable; the phase of the presence of both systemic and local factors, localized malignancy, operable; and fourth when this phase has been superseded by the additional presence of necrobiotic substances producing cell proliferation and metastases.

The monograph provides ample food for thought and argument.

Physiology of Bacteria*

ASIDE from Buchanan and Fulmer's *Physiology and Biochemistry of Bacteria* which has been reviewed in these columns and Stephenson's *Bacterial Metabolism*, there is no other up-to-date work on the subject. The volume under review is the third contribution in this field. The first of the three was written primarily as a reference book and the second emphasized only one phase of bacterial physiology, metabolism. The present writer presents a coordination of the various simplest functions of life with a study of each function and its effect upon the other functions. Attention is devoted primarily to the *necessary* functions of life. To this extent it presents a study of the necessary functions of life as applied to the simplest forms.

Subject matter treated includes endogenous catabolism, energy supply, growth, and the mechanism of death. The volume is a critical summary and review of the literature and appears to be very complete. Undoubtedly it will find a large field of usefulness among bacteriologists and among plant and animal physiologists.

Pathology, Bacteriology and Applied Immunology for Nurses†

A FEW years ago this author published a small handbook on bacteriology and immunology for nurses which was reviewed in this column. The present volume adds a section on pathology. Pathology is divided into two sections, general and special. The first section discusses the causes of disease, the classification of disease and the pathologic changes resultant on the action of the causes. The second section describes the system diseases in sufficient detail so that nurses, technicians and others taking similar types of courses can acquire an adequate general comprehension of what is going on in the patient. The section on pathology as well as that on bacteriology, previously reviewed, is abundantly and excellently illustrated.

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*Physiology of Bacteria. By Otto Rahn, Professor of Bacteriology, Cornell University, Ithaca, N. Y. Pages 438 with 42 illustrations, cloth. P. Blakiston's Son and Company, 1012 Walnut Street, Philadelphia, 1932.

†Pathology, Bacteriology and Applied Immunology for Nurses. By Robert A. Kilduffe, A.B., A.M., M.D., F.A.S.C.P., Director, Laboratories, Atlantic City Hospital; City Bacteriologist, Atlantic City; Serologist, Municipal Hospital for Contagious Diseases, Atlantic City; Pathologist, Atlantic County Tuberculosis Hospital; Serologist, Jewish Seaside Home, Atlantic City, etc. Formerly Major, M.C., U. S. A. Cloth, pages 324. The Bruce Publishing Company, New York, 1931.

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EDITORIAL

The Renaissance of Morgan's Bacillus No. I

THE exact degree of pathogenic importance to be ascribed to the organism first isolated from the feces in cases of summer diarrhea in children by Morgan[1] in 1906 has long been a matter of uncertainty. Relatively recent developments, however, have again brought this organism to the fore and render a review of its present status of interest.

As not only its pathogenic importance but also its bacteriologic identity have been the subject of contention, the present discussion may well be prefaced by a brief description of the organism itself.

The organism first isolated by Morgan in twenty-eight of fifty-eight cases may be described as a slender, gram-negative rod averaging 1 to 3 micra in length when studied in young broth cultures but often exhibiting large and bizarre involution forms in older agar cultures.

Usually slightly motile it may occasionally be immobile. The organism produces indol, most vigorously in young cultures. Gelatin is not liquefied nor milk coagulated. When grown on acetate-agar a brownish discoloration is produced. No growth occurs on citrate agar. Fermentation studies show consistent acid formation with the production of gas in levulose, galactose-peptone water, and glucose, although in the last gas production may be so slight as to be detected only with difficulty in deep stab-glucose agar cultures, and occasionally may even be absent.

As originally described by Morgan[1] and later studied bacteriologically by Morgan[2, 3] and Morgan and Ledingham[4] the organism was grouped in accordance with its fermentation reactions as shown in the table following:[5]

GROUPING OF MORGAN'S BACILLUS IN ACCORDANCE WITH FERMENTATION REACTIONS

	GLUCOSE	MANNITOL	DULCITOL	LACTOSE	SUCROSE
Type I	AG*	O**	O	O	O
Type Flexner	A	A	O	O	O
Type V	A	O	O	O	O
Type Goertner	AG	AG	AG	O	O
Type X	AG	AG	AG	O	AG
Type XII	AG	AG	O	O	AG
Type XIV	AG	AG	O	O	O
Type XV	O	O	O	O	O

*A = Acid; G = gas.
**O = no reaction.

It is quite apparent that the organism is a member of a group of closely related bacteria capable, however, of differentiation by means of their fermentative and also, as shown by other and later workers, their serologic characteristics.

Perhaps the most extensive and thorough bacteriologic study of this group, since its original description, is that made by Thjøtta[6] who concludes from a thorough morphologic and cultural study, as well as from extensive serologic investigations (agglutination and complement fixation tests), that the Morgan bacillus is simply a Bacterium coli of a certain fermenting type and who proposes, therefore, that it be more properly known as Bacillius metacoli (Morgan).

In this view Thjøtta is supported by d'Aunoy.[7]

The bulk of the discussion concerning this organism has not centered upon its bacteriologic identity but upon its ability to produce disease.

Although definitely associated by Morgan, as well as by others, with the production of that form of infectious diarrhea formerly frequently spoken of as "summer complaint" or "summer diarrhea of infants," this assumption has been attacked by many observers.

The principal basis of this disagreement has been two-fold and arises, first, from discordant results of studies of the pathogenicity of the organism for laboratory animals, and, second, upon the failure to demonstrate agglutinin production in the blood of the patients from whom the organism was isolated in connection with processes of which it was probably the cause.

Perhaps because of this uncertainty the organism has not been specifically and consistently looked for but, nevertheless, evidence has been gradually accumulating which purports to confer upon the Morgan bacillus a definite pathogenic

importance and which, moreover, suggests that the organism is not only widely distributed, but also that the pathogenicity for man, of some strains, at least, may be increasing.

The literature on this subject has been extensively reviewed by MacKenzie and Batt[8] from whose paper much of the data to follow has been abstracted.

Thus evidence of the pathogenicity of Morgan's bacillus for mice has been reported by Wilson[9] and for mammals, birds, and reptiles by Lovell.[10]

Efforts to demonstrate the presence of homologous agglutinins where Morgan's bacillus has been isolated from man have met with but inconstant success.

Their occurrence in five of thirteen cases in which the clinical picture was that of paratyphoid fever but blood cultures were positive for Morgan's bacillus has been reported by Havens and Ridgway,[11] and Waaler.[5]

While this angle of the controversy still remains to be elucidated and deserves, as it will doubtless receive, further study, clinical and bacteriologic evidence of the pathogenicity of Morgan's bacillus for man has been the subject of many relatively recent reports.

Dick, Dick, and Williams[12] for example, report an epidemic of enteritis associated with mastoiditis in infants which they regard as due to this organism; and a similar association of pseudomembranous enterocolitis and mastoiditis with the isolation of Morgan's bacillus from the middle ear and heart blood at autopsy is reported by Sutton.[13]

Thirteen cases presenting clinical symptoms in common, characterized by sudden onset, usually with a chill and an equally abrupt termination, are reported by Havens and Ridgway.[11]

These were encountered in Alabama and occurred in adults as well as children.

Positive blood cultures and the presence of homologous agglutinins were found in five cases, Morgan's bacillus being isolated from either the blood or feces in all the cases of the series.

d'Aunoy[7] reports three cases of pyrexia, pyelitis, and colitis in which Morgan's bacillus was isolated, one strain being pathogenic for guinea pigs and white rats, homologous agglutinins being demonstrated in the blood of all three patients. These cases occurred in Louisiana.

An epidemic outbreak in a small rural community in New York State involving ten children from one to fifteen years of age is reported by MacKenzie and Batt[8] in which the primary source of the infection was a contaminated water supply, flies, or direct transmission being responsible for the further spread of the disease.

Five cases of urinary tract infection, four in adults and one in an infant one year old are reported by Waaler,[5] Morgan's bacillus being isolated from all and regarded as the etiologic agent concerned.

Intestinal infections in which Morgan's bacillus is regarded as having some part have been reported by Silverman and Harris[14] and by Mac Gill and Downie,[15] and Pickles,[16] and, finally, Gittins and Hawksby,[17] report the isolation of Morgan's bacillus from the blood and spinal fluid in two cases, one fatal, of meningitis associated with bacteriemia.

The evidence thus briefly summarized suggests not only that, as already recognized, Morgan's bacillus has an extremely wide distribution but also indicates a

very definite pathogenicity for man and suggests in addition that this ability may be on the increase.

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—*R. A. K.*